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Etiology of cat-scratch fever

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EVER since Foshay described cat-scratch fever and made his findings known to others by personal communication, an etiological agent has been sought. The unanimity of opinion that the virus of psittacosis was the infector is remarkable. Despite this, there has been no real evidence produced to implicate this virus. The large volume of literature published in the last few years has not contained any further suggestions as to its etiology, although further attempts to establish its viral origin have been made.

Early investigations established certain facts about the disease,[1, 2, 3] but subsequent studies have failed to disclose the etiological agent, viral or otherwise. It was soon recognized that the cat itself did not have the disease, but was the purveyor, probably from a pool in nature. Mollaret[2] suggested that birds might be the carriers of the causative agent. Attempts to produce the disease in animals failed with the one exception of Mollaret's success in transmitting the disease to a monkey. He did not succeed in transmitting the disease from the monkey to another animal, however.

The infective agent has been sought on the claws of the cat, in his saliva, and about his teeth. No one, including ourselves, has searched in the fur of the animal. This is an oversight which we hope to remedy in the future. Many attempts have been made to isolate a causative agent from the lesion produced in the patient.

A high percentage of patients with cat-

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Table I. Eight cases of cat-scratch fever

Case	*Cat-scratch test*	*Tuberculin test**	*Lygranum test*	*Chromogens*		*Type chromogen*	*Guine*	*pig*
				Smear	*Culture*		*Smear*	*Culture*
347942 E. L.	5 × 5	5 T.U. Negative 10 T.U. +	Negative	Negative	+ Slow growth	Photo-chrome	Negative	Negative
306415 K. R.	Negative	5 T.U. +	Slight +	+	+	Photo-chrome	Negative	Negative
150664 P. C.	5 × 5	10 × 10 5 T.U.	+	+	+ Slow growth	Photo-chrome	Negative	Negative
307744 E. P.	+ Papules	5 T.U. Negative	+ ?	Negative	+	Photo-chrome	Negative	Negative
24540 P. M.	5 × 6	10 × 10 5 T.U.	Negative	1 Negative 2 Negative 3 +	+ + +	Photo-chrome	Negative	Negative
370127	5 × 5	5 T.U. 10 × 10	+	Negative	+ Slow growth	Photo-chrome	Negative	Negative
303059	5 × 6	—	+	+	+ Slow growth	Skoto-chrome	Negative	Negative
275022	Negative	15 × 5	—	Negative	+ Slow growth	Photo-chrome	Negative	Negative

*T.U., Tuberculin units.

†C., incomplete inhibition; Inc., 1 or more colonies in highest concentration; S., 10 colonies or less; PS., 10 to 30 colonies le growth than control.

scratch fever manifested tuberculin sensitivity. This has usually been of low order, but at times of high degree. Erythema nodosum has been reported at the onset of the disease. In children this antigenic reaction suggests either a streptococcal or tuberculous infection as the likely underlying factor. The former was readily ruled out, but the possibility of an acid-fast bacillus infection was not eliminated.

It was not thought that the disease itself was tuberculous, since it was not transmitted to contacts, nor was it familial. As mentioned earlier, the cat itself does not suffer from the disease, but is a purveyor from large pools in nature. Recent world-wide studies have demonstrated the presence in fields and grasses of tuberculin-sensitizing atypical acid-fast bacilli which are not tubercle bacilli. These organisms produce low-grade tuberculin sensitivity in man and animals.[4, 5]

The probability of a viral etiology of the disease seems to have been based largely on the results of skin tests with lygranum (complement fixation). In our series, positive reactions to this antigen were rare. Armstrong and his co-workers[6] suggest that a positive reaction to this test is more closely related to the age of the child than to cat-scratch fever. Further, these workers found positive reactions in normal controls. Their work provides strong evidence that a positive reaction to this test has little probability of being related to cat-scratch fever.

Other viral studies concerned with this disease appear to have been based largely on the hemagglutination of red blood cells with antibodies from the rabbit. The Ohio group[7-10] made the most extensive studies in this field. They succeeded in isolating a virus akin to the herpes virus. No virus has been found by other investigators by inoculation of rabbits, or by examination of presumably contaminated material under the electronmicroscope. Mollaret did find inclu-

pig	Mouse		Enzymes		Neutral red test	Streptomycin†	Bacterial sensitivities paramino salicylic acid (mcg./c.c.)	Isoniazid
Culture	Smear	Culture	Catalase	Peroxidase				
Negative	+	+	+	+	Negative	S. 100 C. 500	Inc. 250	S. 5 C. 100
Negative	Negative	Negative	+	—	Negative	S. 5 C. 100	50 Mcg.	Inc. 100
Negative	Negative	+	+	+	Negative	S. 10 C. 100	S. 5 C. 100	S. 5 C. 100
Negative	Negative	+	+	+	Negative	PS. 10 C. 500	Inc. 250	S. 10 C. 100
Negative	Negative	+	+	+	Negative	S. 25 C. 500	Inc. 250	S. 10 C. 100
Negative	+	+	+	+	Negative	S. 10 C. 100	PS. 5 C. 100	PS. 2.5 C. 10
Negative	Negative	+	+	—	Negative	S. 25 Inc. 500	S. 50 Inc. 250	PS. 10 C. 100
Negative	Negative	Negative	+	—	Negative	S. 10 C. 500	PS. 10 C. 250	Inc. 100

sion bodies in some cells which were considered to be suggestive of viral etiology.

A cat-scratch fever test has been devised which has been used extensively in making a diagnosis of the disease. It is made from an aspirate of the necrotic material caused by the infection. It has not proved a very satisfactory test material. Most patients with the disease show a positive reaction to the intradermal injection with the extract. This is usually of lesser degree than the tuberculin test in the same case. There is no close relationship to the degree of sensitivity shown in the two tests.

Recently, studies of atypical acid-fast organisms have led to the development of antigens made from these organisms. Middlebrook[11] provided us with such an antigen (luciflavin). The recommeded intradermal test dose resulted in a smaller tuberculin reaction than that obtained with Old Tuberculin. Probably a larger dose is indicated.

Another facet of the disease may provide a further opportunity for research. An increasing number of patients with cat-scratch disease have been reported as having encephalitis or meningitis.[12, 13] The demonstration of an acid-fast organism in cerebrospinal fluid would be significant. We have not been able to find a report of such an organism.

A large number of patients with cat-scratch fever have been seen in the clinics of the hospital in recent years. Only a small proportion of these could be included in the study. There was no planned, coordinated study made, and no single group of investigators was involved.

Atypical acid-fast organisms have been reported in the past 10 years in what were thought to be tuberculous lymph nodes. This was mostly before these mycobacteria achieved the status of pathogens, capable of producing disease. Further, there was no classification available as to their tinctorial behavior, enzyme activity, or sensitivities to drugs. Under such circumstances they could

not be included in this paper. It was thought best to present only those cases of which sufficient study had been made to give a complete picture. The number presented was therefore restricted to 8.

As have others during the past few years, we have found increasing numbers of atypical acid-fast organisms in the lymph nodes, the urine, and the gastric washings of patients with or without tuberculous infection. These organisms varied in type and in pathogenicity, but in cat-scratch fever they were invariably of one type.

The many proponents of the viral etiology of this disease might conceivably have found the offending organisms much earlier if routine cultures had been made of the necrotic material. In the past few years their differentiation from tubercle bacilli would have become apparent.

STUDIES

Materials from caseonecrotic lesions, from lymph nodes, and from other tissues of patients with cat-scratch fever were cultured for tuberculosis. Such a procedure appeared mandatory because of the high incidence of positive tuberculin tests found in these patients. No differentiation between tubercle bacilli and atypical acid-fast bacilli was made on direct smear. Cultures were examined in 3 to 4 weeks. Acid-fast organisms were found whose tinctorial propensities precluded their being tubercle bacilli. They proved to be atypical acid-fast bacilli of a chromogen group.

The organisms were subjected to further investigation. Animals were inoculated. No general disease was produced in guinea pigs. Local lesions at the site of inoculation were not uncommon. Mice, on the other hand, all showed some evidence of disease. The reactions in these animals varied from small, localized lesions at the site of injection to more widespread disease, demonstrable only when the mouse was macerated and cultures made from this material. A few chickens were inoculated, but no disease was produced. Hamsters were not used.

The reactions of the organism to two iron porphyrin enzymes, peroxidase and catalase, were studied. They were uniformly positive. No quantitative studies were made.

Bönicke[17] claims the most satisfactory test for sorting out chromogens, particularly photochromogens, is the amidase test. This test was not performed in our cases.

The neutral red test was made in all cases. The earlier tests were done by the cytochemical method, and the later ones by the strip test. The former proved to be the more satisfactory. None of the acid-fast bacilli in the cat-scratch fever cases were found positive to this test. Beeuw[18] attributed the difficulties of producing positive neutral red tests with chromogens by the cytochemical method to an early shift to an acid reaction with the development of a violet color. This test has been generally most useful in establishing the virulence of tubercle bacilli.

COMMENT

The discovery of an acid-fast organism in patients with cat-scratch fever which might have etiological significance was accidental. The approach to our study was certainly in contradistinction to the carefully planned investigation by students of this disease. The frequency with which tuberculin sensitivity was found in these cases warranted culture and other examinations of infected material from the cases studied. Atypical acid-fast organisms were found. It was demonstrated further that there was a sensitivity to an extract of chromogenic acid-fast bacilli.

In the 8 cases studied (Table I), photochromogenic acid-fast bacilli were present in all but one. This exception was in an old case in which the cultures had been carried on for 6 years. The finding of this type of chromogenic bacilli was most significant as it is accepted as a pathogenic organism belonging to a group of great uniformity, and, possibly, to a single species.

SUMMARY

Eight cases of cat-scratch fever are reported in which a photochromogenic acid-fast bacillus was found. The results of en-

zyme studies, animal inoculation, and drug sensitivity tests corroborate the culture findings. No other organism was found in these cases. It therefore seems justifiable to postulate that a photochromogenic acid-fast bacillus will quite likely prove to be the etiological agent in this disease.

REFERENCES

1. Debre, R., Lamy, M., Jeaneret, M. L., Castil, L., et Mazziconaci, A.: La maladie des griffes de chat., Bull. et mém. Soc. Med. hôp. Paris **66:** 76, 1950.
2. Mollaret, P., Reilly, J., Bastin, R., and Tournier, P.: La Découverte du virus de la lymphoréticulose bénigne d'inoculation, Presse méd. **59:** 681, 1951.
3. Debre, R., and Job, J. C.: La maladie des griffes de chat, Acta pédiat. **43:** 1, 1954.
4. Edwards, Lydia, and Krohn, E.: Skin Sensitivity to Antigens Made From Various Acid Fast Bacteria, Am. J. Hyg. **66:** 250, 1958.
5. Palmer, C. E., Krohn, E. F., Manos, N. E., and Edwards, L. B.: Tuberculin Sensitivity of Young Adults in the United States, Pub. Health Rep. **71:** 633, 1956.
6. Armstrong, C., Daniels, W. B., MacMurray, F. G., and Turner, H. S.: Complement Fixation in Cat Scratch Disease Employing Lygranum C.F. as Antigen, J. A. M. A. **161:** 149, 1956.
7. Dodd, M. G., Graber, C. D., and Anderson, G.: Hemagglutination of Rabbit Erythrocytes by Pus From Cases of Cat Scratch Fever, Proc. Soc. Exper. Biol. & Med. **102:** 556, 1959.
8. Josiukas, T., Roy, T. E., and Boyd, G. L.: The Middlebrook-Dubos Hemagglutination Test for Tuberculosis in Children and Adults, J. Clin. Invest. **33:** 1415, 1954.
9. Turner, W., Bigley, N. J., Dodd, M., and Anderson, G. E.: Hemagglutinating Virus Isolated From Cat Scratch Disease, J. Bact. **80:** 430, 1959.
10. Brown, Milton: Research Project, Connaught Laboratories, Tuberculin Production in Atypical Acid Fast Bacilli (not published).
11. Middlebrook, G.: Diagnostic and Biological Problems of Isoniazid Resistant Tubercle Bacilli, Bull. Internat. Union Against Tuberculosis **179:** 1956.
12. Paxon, E. M., and McKay, R. J.: Neurologic Symptoms Associated With Cat Scratch Disease, Pediatrics **20:** 13, 1957.
13. Stevens, H.: Cat Scratch Fever Encephalitis, A. M. A. Am. J. Dis. Child. **84:** 218, 1952.
14. Debre, R., Rogart, L., van Thieffry, T., and Anthuris, M.: Accident nervoux de la maladie des griffes de chat, Bull. Acad. nat. méd. **136:** 454, 1952.
15. Runyon, E. H.: Anonymous Mycobacterium in Pulmonary Disease, M. Clin. North America **43:** 73, 1959.
16. Runyon, E. H.: Tuberculosis Co-operative Study, Am. Rev. Tuber. **72:** 866, 1955.
17. Bönicke, R.: The Classification of "Atypical" Mycobacteria by Determination of Their Varying Acylamidase Metabolic Performances, Ger. M. Monthly **5:** 232, 1960.
18. Beeuw, H.: The Use of the Neutral Red Test in Culture Media for the Differentiation Between Mycobacterial Tuberculosis and Atypical Mycobacteria, Proc. Tuberculosis Research Council (Holland) **46:** 1959.

Infection due to a "related" Vibrio

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RECENT reports have directed attention to the "related" Vibrio as a human pathogen. King[1] reported upon 3 infants and 1 child from whom this organism had been isolated. Wheeler and Borchers[2] have recently added 4 cases, in 3 of which a "related" Vibrio was present in the blood. The "related" vibrios described in these reports appear as small, slender, curved, gram-negative rods with frequent longer spiral forms but are indistinguishable, morphologically, from the other vibrios. They are actively motile with a single flagellum. These are microaerophilic organisms, which grow at 37° C. with optimum growth occurring at 42° C. In a candle jar this same Vibrio will produce small punctiform, nonhemolytic colonies on rabbit blood agar in 24 hours and some growth on MacConkey's agar. No growth will occur on SS (Salmonella-Shigella) agar. Since other biologic tests frequently used to distinguish different vibrios are quite variable among "related" vibrios, serologic methods are best for definitive identification. Recently, a "related" Vibrio was isolated from the blood of an infant with diarrhea who was in the St. Louis Children's Hospital. Since only 8 cases of infection from this cause have been reported in human beings, this report may add further information about this newly recognized disease.

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CASE REPORT

Y. W., a 2-month-old female Negro, was admitted to the St. Louis Children's Hospital for diarrhea of 2 weeks' duration. Her stools had been blood streaked intermittently for the previous 4 days, but contained increasing amounts of bright red blood during the 36 hours prior to admission.

It was stated that the patient was a full-term infant with normal delivery. The birth weight was 6 pounds, 1 ounce. The mother had had recurrent infections of the urinary tract during her pregnancy. The patient did well on a cow's milk formula. At 5 weeks of age, she accompanied her mother and 2 siblings, 21 months and 3 years of age to Mississippi to visit maternal relatives. Her formula during the visit was prepared from canned cow's milk and boiled well water. After 10 days in Mississippi, she developed loose green stools. Neither her siblings nor her relatives became ill. The diarrhea then became progressively worse for the 4 days prior to hospitalization. During the 36 hours

preceding admission to the hospital, there were large amounts of bright red blood in the stools.

Physical examination. On admission the physical examination revealed a rectal temperature of 37° C., a pulse rate of 200 per minute, and respirations of 34 per minute. The infant was crying constantly and moving about in apparent acute distress. Her skin was dry and had poor turgor. The anterior fontanel was depressed. There was generalized abdominal guarding, but no rigidity and no masses were palpable. Rectal examination revealed bright red blood with mucus present on the examining finger. The remainder of the physical examination was within normal limits. A tentative diagnosis of intussusception was made at the time of admission to the hospital.

Laboratory data. On admission the hemoglobin was 11.6 Gm. per 100 ml. of blood, and there were 4.33 millio.. red blood cells per cubic millimeter, and a hematocrit of 31 per cent. The peripheral white blood count was 16,240 per cubic millimeter, with a differential blood cell count of 2 eosinophils, 8 stab forms, 25 segmented neutrophils, 53 lymphocytes, and 12 monocytes. The platelets appeared to be adequate in number. The urinalysis was normal. Blood serum determinations revealed the carbon dioxide content to be 18.2, chloride 97.9, sodium 128, and potassium 3.5 mEq. per liter; nonprotein nitrogen 23.1 mg. per 100 ml., total protein 4.55 Gm., and albumin 3.65 and globulin 0.9 Gm. per 100 ml. A throat culture revealed a predominance of pneumococci. A stool culture showed no Salmonella or Shigella, and the *Escherichia coli* were negative with antisera against enteropathogenic Types 0111, 055, 0127, 026, 086, 0119, 0125, 0126, 0128, 024, 044, 075, and 0112. No parasites or ova were found in the stool.

Typhoid O and H and paratyphoid A and B agglutinins were not demonstrable in the patient's blood serum. Blood cultures after 24 hours revealed an organism which was later identified as a "related" Vibrio.

Immediately after admission the patient received parenteral fluids and was placed on chloramphenicol, 30 mg. per kilogram of body weight per 24 hours. She improved rapidly, remained afebrile, and was discharged after 48 hours in the hospital. The chloramphenicol was continued by her private physician for a total of 6 days. The patient has been well with no relapse for 6 months.

Bacteriology. The "related" Vibrio was isolated from whole blood cultured in thioglycollate broth at 37° C. An aliquot of the original sample cultured concurrently at 37° C. in tryptose phosphate broth and combined with beef heart infusion agar in the form of a pour plate failed to demonstrate the organism. The morphology of this organism was predominately spirillar at 24 and 48 hours of growth (Fig. 1), but by 96

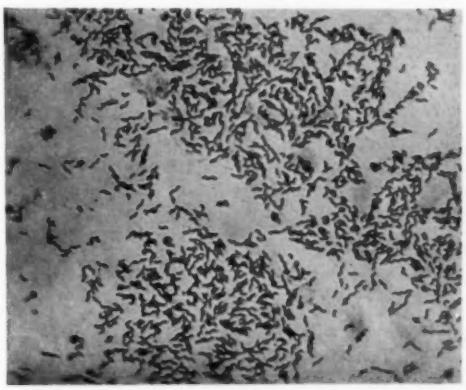

Fig. 1. Morphology of "related" Vibrio at 24 hours' growth (×1200).

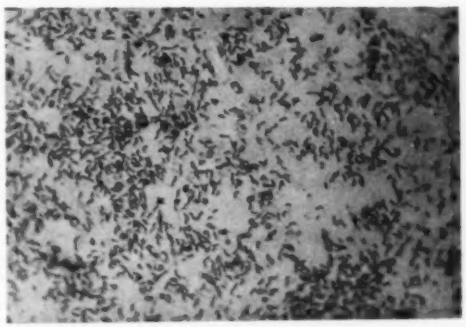

Fig. 2. Morphology of "related" Vibrio at 96 hours' growth (×1200).

Table I. Biologic characteristics of the Vibrio isolated from Patient Y. W.

Media	*Reaction*
Pigment	None
Catalase	1+
Oxidase	Positive
Glucose	No reaction
Xylase	No reaction
Mannitol	No reaction
Lactose	No reaction
Sucrose	No reaction
Maltose	No reaction
Citrate	Negative
Urea	Negative
Nitrate	Positive
Indol	Negative
Methyl red	Negative
Voges-Proskauer	Negative
Gelatin	No reaction
Litmus milk	Negative
Hemolysis	Negative

Table II. Serologic studies on Vibrio isolated from Patient Y. W.*

Organism	*Strain*	*Animal source*	*Agglutination titer of Vibrio (Y. W.)*†
Vibrio fetus	1134	Human	0
Vibrio fetus	1154	Human	0
Vibrio fetus	543	Ovine	0
Vibrio fetus	P-1	Bovine	0
Vibrio fetus	RL-51	Bovine	0
Vibrio bulbus	DL-fashion	Bovine	0
"Related" Vibrio	1133	Human	1,280
"Related" Vibrio	2737	Human	0
"Related" Vibrio	3695	Chicken	160

*The antisera used in these tests are produced in rabbits for the strain-specific organism listed in the left and middle columns.

†Figures given are reciprocals of highest titer of agglutination.

hours of cultivation, the comma form was frequent (Fig. 2). The organism was gram negative, flagellated, motile, and grew best under microaerophilic conditions. Other characteristics of this organism are listed in Table I. It was considered pathogenic and not a contaminant of the blood culture.

The serologic identification of the Vibrio was done by Miss Elizabeth O. King of the Communicable Disease Center of the United States Public Health Service in Chamblee, Georgia. The results are listed in Table II. Since the parents would not allow a blood sample to be obtained from the patient after discharge, no serologic studies were done.

DISCUSSION

Recent medical literature indicates that there has been an apparent increase in the number of human infections with *Vibrio fetus*[3-7] and "related" vibrios.[1, 2] Since 1909[8] these organisms have been known to produce disease in animals, but the association of the human disease with these vibrios was not recognized until recently. Wheeler and Borchers[2] reviewed all previously reported cases of "related" Vibrio infections in human beings. The clinical courses were similar to that of our patient. Of the 8 previously reported patients, 6 of them were young infants. All of them had diarrhea, and 6 had blood in the stools. In most, the diarrhea had been present only about 1 to 3 weeks, but in 2, diarrhea persisted for possibly 9 months. Six of the 8 patients had been vomiting, 2 were febrile at the time they were examined. The patients did not have acidosis, but one infant was dehydrated and required parenteral fluid as did our infant. The patient in our report responded to chloramphenicol administered orally for 8 days and there was no evidence of relapse after 6 months. Successful treatment of other patients has been reported with sulfisoxazole, neomycin, streptomycin, tetracycline, and penicillin as well as chloramphenicol. It was suggested by Wheeler and Borchers that without specific treatment it may be possible that these vibrios cause a relapsing enteritis, which occurred in one of their patients.

Inflammatory lesions, primarily in the jejunum of calves and sometimes in the entire small intestine, have been produced by *Vibrio jejuni*.[9-11] Wheeler and Borchers state that the jejunum and upper half of the ileum were the primary sites of inflammation and hemorrhagic necrosis found in the autopsy of an adult from whom a "related" Vibrio was isolated. There have been no pathologic observations in infants or children with "related" Vibrio infections.

The organism was isolated from the whole blood of our patient, as in 7 of the previous 8 cases reported. The reason this organism may not have been isolated more frequently is because of the peculiar conditions necessary for its growth, such as incubation at 42° C. under increased carbon dioxide. Overgrowth of coliform organisms could easily prevent its isolation from stool culture.

Wheeler and Borchers state that vibrios have been isolated from the livers and gall bladders of chickens with avian vibrionic hepatitis. These are said to be biochemically indistinguishable from "related" vibrios in human beings. It is interesting that the species isolated from our patient was agglutinated in a high titer by antiserum prepared against a human strain, and also in a lower titer by antiserum prepared against an organism isolated from a chicken. The mother stated she had dressed several chickens while in Mississippi, but did not recall any of the chickens having been ill before slaughter. The father has been employed in a packing house for the past 2 years as a handler and transporter of frozen fish, chicken, pork, beef, and mutton but he has never had contact with fresh meat. There were no pets in their home. Serum specimens obtained from the father and mother had no demonstrable antibodies against the strains of "related" vibrios found in human beings. Unfortunately, the original isolate in our laboratory as well as the subculture at the United States Public Health Service Laboratory was not viable, so the parents' serum could not be tested against this organism.

SUMMARY

1. A "related" Vibrio was isolated from the blood of an infant with bloody diarrhea.

2. The unusual growth requirements of this organism may have prevented its isolation previously and precluded its association with the disease in human beings.

3. The eight cases previously reported are briefly reviewed and a possible epidemiologic association with Vibrio infection of chickens is raised.

We wish to thank Dr. Seymour Schlansky for allowing us to report upon his patient. We are indebted to Miss Elizabeth O. King for confirming the identity of this organism and for her serologic studies on the patient's parents.

REFERENCES

1. King, Elizabeth O.: Human Infections With Vibrio fetus and a Closely Related Vibrio, J. Infect. Dis. **101:** 119, 1957.
2. Wheeler, Warren E., and Borchers, Justine: Vibrionic Enteritis in Infants, A. M. A. J. Dis. Child. **101:** 60, 1961.
3. Spink, W. W.: Human Vibriosis Caused by Vibrio fetus, J. A. M. A. **163:** 180, 1957.
4. Ward, B. Q.: The Apparent Involvement of Vibrio fetus in an Infection of Man, J. Bact. **55:** 113, 1948.
5. Hood, M., and Todd, J. M.: Vibrio fetus—A Cause of Human Abortion, Am. J. Obst. & Gynec. **80:** 506, 1960.
6. Jackson, J. F., Hinton, P., and Allison, F.: Human Vibriosis. Report of a Patient With Relapsing Febrile Illness Due to Vibrio fetus, Am. J. Med. **28:** 986, 1960.
7. Kahler, Richard L., and Sheldon, Hintington: Vibrio fetus Infection in Man, New England J. Med. **262:** 1218, 1960.
8. MacFadyean, J., and Stockman, S.: Report of the Departmental Committee Appointed by the Board of Agriculture and Fisheries to Inquire Into Epizootic Abortion, London, 1909, His Majesty's Stationery Office, part I.
9. Jones, F. S., and Little, R. B.: The Etiology of Infectious Diarrhea (Winter Scours) in Cattle, J. Exper. Med. **53:** 835, 1931.
10. Jones, F. S., and Little, R. B.: Vibrionic Enteritis in Calves, J. Exper. Med. **53:** 845, 1931.
11. Jones, F. S., Orcutt, M., and Little, R. B.: Vibrios (Vibrio jejuni N. Sp.) Associated With Intestinal Disorders of Cows and Calves, J. Exper. Med. **53:** 853, 1931.

Corticosteroid treatment for measles encephalitis

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IT IS common practice to use corticosteroid therapy in the treatment of measles encephalitis. Appelbaum and Abler[1] treated 17 patients with measles encephalitis with ACTH. Their dose ranged from 5 to 80 mg. per day and varied from 1 to 14 days in duration. They reported that no patients died and that 14 were discharged from the hospital without sequelae. Only one patient had permanent neurological damage. Allen[2] treated 10 patients with measles encephalitis with an intensive standardized dose of corticosteroid and all patients recovered within 14 days and none was noted to have sequelae at examination 6 months later. However, controlled studies proving the efficacy of corticosteroid treatment in this condition have not been reported. This article presents the results of the treatment of 38 patients with measles encephalitis.

METHOD

Patients accepted for the study had a history of measles within the preceding 15 days and signs of meningeal irritation, mental confusion, lethargy, convulsions, paralysis, and/or coma. The treatment of the 32 patients in the study was determined by the last digit of the admission number. The even numbered patients received corticosteroids and the odd numbered patients were treated symptomatically. We know of one instance where the last digit was changed by the admitting resident who wished to control the form of therapy of his patient. This was done by calling the clerk and timing the admission to get an even number. As a consequence this patient was placed in the corticosteroid-treated group instead of in the control group.

Corticosteroid therapy followed that set forth by Allen and consisted of an immediate dose of 100 units of hydrocortisone hemisuccinate given intravenously, followed by another 100 units during the first 24 hours. Cortisone, 300 mg., was given intramuscularly on the first day, 150 mg. the second day, and 75 mg. the third day. ACTH, 40 units, was administered after the first 24 hours, 20 units the third day, and 10 units the fourth day. As suggested by Allen, a dose of penicillin of 1.2 to 6 million units per day and one of chloramphenicol of 100 mg. per kilogram of body weight per day were administered.

The control group was treated symptomatically. Fluids were given intravenously if necessary and antibiotics if evidence of bacterial disease was noted.

The same nursing care was given each group. The nurses in charge were experi-

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Table I. Results

Findings	Controlled study: Corticosteroid therapy (16 patients) %	Controlled study: Corticosteroid therapy (16 patients) No.	Controlled study: Symptomatic therapy (16 patients) %	Controlled study: Symptomatic therapy (16 patients) No.	Noncontrolled study: Corticosteroid therapy (3 patients) No.	Noncontrolled study: Symptomatic therapy (3 patients) No.
Delirium or coma	56	9	56	9	1	1
Lethargy	31	5	31	5	1	2
Abnormal spinal fluid	87	14	81	13	2	2
Convulsions	62	10	45	7	1	2
Deaths	12	2	0	0	0	0
Paralysis on discharge	45	7	25	4	1	1
Average days to improve	3.3*		3.5		2.6	3.6
Range	1 to 8		1 to 16		2 to 3	3 to 4
Average days in hospital	10.3		8.5†		11.3	8.6
Range, days	4 to 20		2 to 26		8 to 15	6 to 10

*Excludes 2 patients who died on the fifth and ninth hospital days, respectively.

†Includes 2 patients who signed out of hospital against advice.

enced in the care of patients with contagious disease and were expert with the mechanical respirator, maintenance of airway, and other nursing procedures necessary for the survival of patients in coma.

Gavage feedings were not permitted during the acute phase of the disease and, if the patients were not able to take food by mouth, fluid balance was maintained by the intravenous route. One patient in coma for 16 days was given food by gavage after the sixth day of coma. Six other patients are included since half were treated with corticosteroid and half were not, although the treatment for these patients was selected at random and prior to the establishment of the controlled study.

RESULTS

The average length of time before marked improvement was recorded was about 3.5 days in each group; the average length of hospital stay was 8.5 days* for the control group and 10.3 days in the corticosteroid-treated group. Seven of 16 patients treated with corticosteroids left the hospital with paralysis as did 4 of 16 patients in the control group. Three of the 7 treated patients who had neurological damage at the time of discharge were followed for 2 years and were well at that time. We were not able to follow the 4 remaining patients. Three of the 4 control patients who had paralysis on discharge were followed for 2 years and were well at this time. Contact was lost with the remaining patient. No deaths were recorded in the control group, but 2 deaths were recorded in the corticosteroid-treated group. At postmortem examination, one child had demyelinating encephalitis and pneumonia and the other child had a peptic ulcer with severe hemorrhage as well as demyelinating encephalitis and pneumonia. It was thought that the hemorrhage and shock following the ulcer contributed to his death.

*Includes 2 patients who signed out of the hospital against advice.

SUMMARY

The effectiveness of corticosteroid therapy in measles encephalitis was observed in a clinical study of 38 patients; 32 of these comprised a controlled study group.

There was no apparent benefit related to corticosteroid therapy.

There were 2 deaths; each of these patients received corticosteroid therapy.

REFERENCES

1. Applebaum, E., and Abler, C.: Treatment of Measles Encephalitis With Corticotropin, A. M. A. J. Dis. Child. 92: 147, 1956.
2. Allen, John E.: Pediatrics 20: 87, 1957.

Prospects for measles immunization with reference to the relationship between distemper and measles viruses

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MEASLES has been the common experience of mankind for such a long time that most people have come to accept it as an inevitable, not especially dangerous, nuisance. Even among the medical profession, where measles is a recognized source of troublesome complications, its dangerous potentialities are sometimes underestimated.

But it is increasingly evident, particularly in countries with relatively high standards of hygiene, that measles is one of the most serious of the common diseases of childhood. True, both its severity and the mortality from it have declined in recent decades with the use of sulfonamides, antibiotics, and gamma globulin. However, some complications of measles do not usually respond to antibiotic treatment, and mortality still is considerable. Even in the United States, where between 1934 and 1955 the death rate from measles dropped from 5.5 to 0.2 per 100,000, there were still 490 deaths reported in 1958.[1] In other countries, for example Chile, where the 1934 death rate from measles was 36.6 per 100,000, the decline in mortality has been much less dramatic; as late as 1956, the measles death rate of children between 1 and 4 years of age was 35.6 per 100,000.[2]

Among the dangerous complications of measles are staphylococcal pneumonia, which often is fatal, interstitial giant cell pneumonia, the etiology of which was unknown until the recent report of its association with measles virus,[3] and encephalitis, which frequently results in death or permanent disability. Recent electroencephalographic studies have shown that frank clinical measles encephalitis represents only a fraction of the neurological disturbances potentially associated with measles.[4]

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The need for some effective measles-control procedure can scarcely be denied. Indeed, efforts to control measles by specific immunization antedate the isolation of the measles virus. Deliberate exposure of children long has been the empirical practice in many parts of the world. In the United States before World War I, secretions from patients with measles were used by Herrmann[5] to induce immunizing infections in infants with low parenterally acquired resistance. In 1941, Shaffer and colleagues[6] reported their experience with what was believed to be chick embryo–propagated measles virus. Although inoculated children and monkeys developed mild but definite measles symptoms, results on the whole were inconclusive and suffered from the lack of a method for demonstrating infectivity, virus multiplication, and antibody development. Not until antibiotics and tissue culture joined forces was sustained progress possible. Pioneers of this work were Enders and his collaborators[7-12] in the United States and Zhdanov and Fadeyeva[13] in the Union of Soviet Socialist Republics.

As an alternative to a specific immunizing agent, the possibility of a relationship between canine distemper and measles viruses[14-17] has suggested to some investigators the use of modified distemper virus to protect children against measles. But, to date, results of experiments with distemper virus have been equivocal.[18-21]

In addition to presenting here a summary of our findings concerning an experimental live measles-virus vaccine in children, it is proposed to present some laboratory evidence of the dissimilarity of distemper and measles viruses.

STUDIES WITH DISTEMPER VIRUS

In our hands, results of several cross-immunologic experiments, in a number of animal species and in tissue culture, failed to confirm a relationship between the two viruses. In these experiments the distemper agents used were the Lederle chick embryo–adapted strain, which causes typical lesions on the chorioallantoic membrane,[22] the Lederle virulent strain, which kills susceptible ferrets in 8 to 14 days, and the Snyder Hill strain, virulent for dogs. The measles agent used was the Edmonston strain,[7] adapted to grow either in chick embryo or in HeLa cell or monkey kidney tissue culture. Antibody levels of the experimental animals were titrated by serum neutralization methods, in chick embryo for distemper and in HeLa cells for measles. In one experiment measles antibodies were also determined by the complement-fixation method.

In an experiment with dogs, a litter of nine 10-week-old puppies was used, as shown in Table I. Six animals were vaccinated subcutaneously with a 2 ml. dose of a 30 per cent distemper-infected chorioallantoic membrane suspension. Five weeks later, 3 of the vaccinated and the 3 unvaccinated animals were challenged intravenously with a 1.5 ml. dose of a 10 per cent dog-brain suspension containing about 1,500 LD_{50} of the Snyder Hill strain of distemper virus. Throughout the observation period, all 6 vaccinated animals, including those challenged, remained normal. The other 3 developed signs of distemper; subsequently, one recovered, one suffered a prolonged illness and was killed when moribund 44 days after challenge, and the third died of distemper on the twenty-fifth day.

The distemper responses antibody are shown in Table II. All animals were bled at

Table I. Schedule of immunization and outcome of challenge of puppies with canine-distemper virus

Puppy No.	*Immunization status*	*Challenge outcome*
1	Vaccinated	Not challenged
2	Vaccinated	Not challenged
3	Vaccinated	Not challenged
4	Vaccinated	Remained normal
5	Vaccinated	Remained normal
6	Vaccinated	Remained normal
7	Not vaccinated	Sickened—killed 44th day
8	Not vaccinated	Sickened—recovered
9	Not vaccinated	Sickened—died 25th day

Table II. Canine-distemper antibody titers of puppies before vaccination and before and after challenge with canine-distemper virus

Puppy No.	*Prevaccination*	*Postvaccination (4 to 5 weeks)*	*Postchallenge (8 to 10 weeks)*
1	<1:5	1:1,400	—
2	<1:5	1:1,200	—
3	<1:5	1:1,400	—
4	<1:5	1:1,400	1:1,400
5	<1:5	1:940	1:280
6	<1:5	1:1,600	1:8,600
7	<1:5	<1:5*	1:330†
8	<1:5	<1:5*	1:400
9	<1:5	<1:5*	1:10‡
Control: Distemper-immune dog serum: 1:1,000 to 1:7,000			

*Not vaccinated.
†Serum sampled thirty-fifth day.
‡Serum sampled twenty-first day.

the time of vaccination and again 4 to 5 weeks later. In addition, challenged animals were bled a third time 8 to 10 weeks after the challenge, except for the one that died, which was bled on the twenty-first day, and the one that was killed, bled on the thirty-fifth day. Results of serum-neutralization tests indicate that, with the exception of the one that died, all vaccinated animals developed appreciable distemper antibodies, titers being of the same order before and after the challenge.

In contrast, as Table III shows, neither serum-neutralization nor complement-fixation tests detected any measles antibodies in any of the sera.

In an experiment with a different animal species, twelve 7-month-old roosters, each weighing approximately 8 pounds, were inoculated according to the schedule shown in Table IV, 6 receiving canine-distemper and the other 6 receiving measles virus. On days 0 and 21, each bird received intramuscularly, in each of 4 sites, a 2.5 ml. dose of virus-adjuvant mixture* and intravenously a single 1.0 ml. dose of adjuvant-free virus suspension, and on day 7 another intravenous 1.0 ml. dose of the adjuvant-free virus suspension. The distemper-virus preparation was either a 10 per cent or a 20 per cent chorioallantoic membrane suspension; the measles-virus preparation, an undiluted tissue culture fluid. All birds were bled at the beginning and end of the 31 days' experiment.

*Virus-adjuvant mixture: 5 parts virus suspension, 1 part Arlacel (mannide monooleate), and 4 parts Bayol F (mineral oil).

Results of neutralization tests for distemper and measles antibodies are shown in Table V. It is obvious that response was specific to the antigen received. The 6 birds inoculated with distemper virus developed distemper antibodies, and 5 of the 6 inoculated with measles virus developed measles antibodies.

The next two experiments were conducted in ferrets. In the first one, 12 groups of 4 ferrets were inoculated intraperitoneally. Ten of the groups were inoculated each week for 4 weeks, Groups 1 through 5 re-

Table III. Measles antibody titers of puppies before vaccination and before and after challenge with canine-distemper virus

Puppy No.	*Prevaccination*		*Postvaccination (4 to 5 weeks)*		*Postchallenge (8 to 10 weeks)*	
	*CF**	*NI†*	*CF*	*NI*	*CF*	*NI*
1	<1:4	0	<1:4	0	—	—
2	<1:4	0	<1:4	0	—	—
3	<1:4	0	<1:4	0	—	—
4	<1:4	0	<1:4	0	<1:4	0
5	<1:4	0	<1:4	0	<1:4	0
6	<1:4	0	<1:4	0	<1:4	0
7	<1:4	0	<1:4‡	0‡	<1:4§	0§
8	<1:4	0	<1:4‡	0‡	<1:4¶	0¶
9	<1:4	0	<1:4‡	0‡	<1:4¶	0¶
Controls: Distemper-immune dog serum; Measles-immune monkey serum	CF: <1:4; 1:128-1:256		NI: 0; 315-3,150			

*CF = complement-fixation.
†NI = neutralization index.
‡Not vaccinated.
§Serum sampled the thirty-fifth day.
¶Serum sampled the twenty-first day.

Table IV. Schedule of hyperimmunization of roosters with canine-distemper or measles virus

Day	*Inoculum*		*Dose (ml.)*	*No. of Injections*	*Route†*	*Serum sampled*
	*Virus and adjuvant**	*Virus only*				
0	×		2.5	4	IM	×
		×	1.0	1	IV	
7		×	1.0	1	IV	
21	×		2.5	4	IM	
		×	1.0	1	IV	
31						×

*Virus-adjuvant mixture: 5 parts virus suspension, 1 part Arlacel, 4 parts Bayol F.
†IM = intramuscular; IV = intravenous.

ceiving a 1 ml. dose of measles virus, grown in monkey kidney, representing $10^{4.3}$ to 10^{5} TCD_{50}, and Groups 6 through 10 receiving a 1 ml. dose of tissue culture suspension of normal kidney. The other two groups received a single injection of 0.5 ml. of a 30 per cent suspension of chick embryo–adapted distemper virus. Blood samples were taken from each animal by cardiac puncture, before the first and 12 days after the fourth inoculation, and pre- and postinoculation sera were pooled by group. With the exception of one which died as a result of bleeding, all animals remained well until challenged.

Immediately after the last bleeding, Groups 1 through 10 were challenged with serial tenfold dilutions of virulent canine-distemper virus, a 1 ml. dose of each dilution being injected intraperitoneally into one group which had been immunized with measles virus and one group which had received normal-kidney tissue. The two groups immunized with modified distemper virus were challenged with 1 ml. of the highest concentration of virulent distemper virus.

Table VI presents the pre- and postimmunization levels of antibody against both measles and distemper viruses. Animals which received measles virus developed neither measles nor distemper antibodies, whereas those which received distemper virus developed high titers of homologous antibody but no antibodies against measles.

As shown in Table VII, a single injection of modified distemper virus immunized against the highest distemper-challenge concentration, but immunization with measles virus did not protect against even one ferret LD_{50} of distemper. Since the failure of measles-immunized animals to develop detectable measles antibodies could be construed as the reason for their failure also to resist distemper challenge, another experiment was performed to determine how the presence of measles antibodies would affect susceptibility to distemper virus.

For this experiment, 4 groups of 3 ferrets were inoculated according to the schedule in Table VIII. Groups 1 and 2 received a HeLa-grown measles-virus suspension, administered as follows: on days 0 and 14, in each of two sites, a 2 ml. intramuscular injection of a mixture of virus and adjuvant and a single 1 ml. intraperitoneal injection of virus suspension alone; on days 7 and 21, a single 1 ml. intraperitoneal injection of virus suspension. Groups 3 and 4 received a normal HeLa cell preparation in place of the virus suspension. Two additional ferrets were immunized on day 0 with modified distemper virus. All animals were bled on days 0, 21 and 31, and sera of each bleeding date were pooled by group. After the third bleeding, the animals were challenged with virulent distemper virus.

As Table IX shows, one of the measles-immunized groups, one virus-free control group, and the distemper-immunized animals received 10,000 ferret LD_{50}, and the

Table V. Antibody titers of roosters hyperimmunized with canine-distemper or measles virus

Rooster No.	*Immunized with**	*Antibody titers*			
		CD†		*MV‡*	
		Pre	*Post*	*Pre*	*Post*
1	CD	<1:5	1:940	0	0
2	CD	<1:5	1:125	0	0
3	CD	<1:5	1:420	0	0
4	CD	<1:5	1:240	0	0
5	CD	<1:5	1:55	0	0
6	CD	<1:5	1:55	0	0
7	MV	<1:5	<1:5	0	0
8	MV	<1:5	<1:5	0	315
9	MV	<1:5	<1:5	0	315
10	MV	<1:5	<1:5	0	315
11	MV	<1:5	<1:5	0	>315
12	MV	<1:5	<1:5	0	>315

*CD = canine-distemper virus; MV = measles virus.

†Titers represent serum dilution neutralizing virus in 50 per cent of inoculated chick embryos.

‡Neutralization index.

Table VI. Measles and canine-distemper antibody titers of ferrets immunized with measles virus or modified distemper virus and of nonimmunized ferrets

Group	*Neutralizing antibody titers**			
	MV†		*CD‡*	
	Pre	*Post*	*Pre*	*Post*
1 to 5 (MV immunized)	<4	<4	<5	<5
6 to 10 (nonimmunized)	<4	<4	5; <5	5; <5
11 (CD-immunized)	<4	<4	<5	1,200
12 (CD-immunized)	<4	<4	<5	830
Control immune serum:				
MV		140		20
CD		<4		4,000

*Expressed as reciprocal of dilution.

†MV = measles virus.

‡CD = canine-distemper virus.

Table VII. Response to challenge with virulent canine-distemper virus of ferrets immunized with measles or modified distemper virus and of nonimmunized ferrets

Group	*Mortality ratio* following CD† challenge*					
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	*End point*
1 to 5 (MV‡ immunized)	4/4	4/4	4/4	4/4	3/4	$>10^{-5.3}$
6 to 10 (Nonimmunized)	4/4	4/4	4/4	4/4	1/4	$10^{-4.7}$
11 (CD-immunized)	0/3§					
12 (CD-immunized)	0/4					

*Number dead/number challenged.

†CD = canine-distemper virus.

‡MV = measles virus.

§One animal died before challenge.

other two groups 1,000 such doses of distemper virus. Although, with the use of adjuvant, demonstrable measles antibodies were elicited in the ferrets inoculated with measles virus, no distemper antibodies developed and all those animals succumbed to distemper challenge. In contrast, immunization with modified distemper virus induced both distemper antibodies and immunity to challenge, but did not induce measles antibodies.

To determine distemper and measles antibodies in sera of human patients with measles 13 paired acute and convalescent serum samples* and 3 single convalescent samples* collected during a 1947 epidemic of measles in Cleveland were used. They had been stored in a dry-ice cabinet until tested in our laboratories. Clinical signs observed in each patient during the acute phase of the disease and neutralization titers of sera of acute and convalescent phases are shown in Table X. Starting dilutions differed because of differences in the amounts of serum available. There is clearly no correlation between the appearance of measles and distemper antibodies in any of the patients. Patient No. 16 shows no significant increase in measles antibodies, in agreement with the diagnosis of "no clinical measles." The highest levels of distemper antibody, between 1:10 and 1:30, were found in Patients No. 1 and No. 16 and in the measles-immune monkey controls. In interpreting distemper-neutralization tests of dog serum in chick embryos, titers of less than 1:20 are not considered significant, since animals with such or even slightly higher titers have been found fully susceptible to challenge.[23] As illustrated by the control distemper-immune dog serum, actively immune dogs frequently have titers of 1:1,000 or greater. This serum, incidentally, was devoid of measles antibodies. The low levels of measles antibody in both serum samples of Patient No. 10 may indicate that his rash was caused by some agent other than measles virus. Judging by their convalescent phase titers, all the other patients did have clinical measles, perhaps including the 3 from whom acute specimens were not available. Nevertheless, none of them had significant distemper antibodies.

In the next experiment, the effect of homologous and heterologous antisera on the multiplication of measles and canine-distemper viruses in tissue culture was investigated. Results are summarized in Table XI. Measles virus was grown in HeLa cell tissue culture tubes in the presence of Eagle's medium[24] supplemented by one of the following: 5 per cent normal horse serum, 5 per cent measles-immune monkey serum, or 5 per cent distemper-immune dog serum. Tubes were incubated at 37° C. for 14 days, and pooled fluids from each group were titrated in HeLa cell cultures.

Other tubes, of chick embryo culture, were renewed, one group with Medium 199 alone[25] and the others with Medium 199 plus one of the following: 2.5 per cent normal dog serum, 2.5 per cent control distemper-immune dog serum, or 2.5 per cent control measles-immune monkey serum. All these tubes were inoculated with tissue culture–adapted distemper virus[26] and incubated at 37° C. for 4 days. Tissues and fluids from each group were harvested, ground in a Ten Broeck grinder, and titrated on the chorioallantoic membrane of 7-day-old chick embryos.

It can be observed that the growth of measles virus was significantly inhibited (3 logs difference in titer) by the homologous serum and not at all by the heterologous serum, whereas the growth of distemper virus was inhibited by the presence in the medium of distemper-immune serum and not by the presence of measles antiserum.

In a final experiment, the distemper-measles virus antibody pattern of measles-immunized monkeys was determined. Antibody titers are shown in Table XII. One monkey developed antibodies to measles virus a week after immunization, but died of pneumonia on the thirteenth day. The other monkeys developed measles antibodies

*Provided by Dr. A. B. Sabin of Children's Hospital, Cincinnati, Ohio.

by the second week and maintained high titers until the end of the 6 weeks' observation period. Two animals (Nos. 4 and 5) developed nonsignificant distemper titers, and none of the others developed any distemper antibodies despite their elevated levels of measles antibody.

It is evident that our results are at variance with those of others who suggest a relationship between measles and distemper viruses. Results, with the strains used in our studies, indicate little similarity between the two agents, and any relationship seems remote. We, therefore, consider it questionable that effective protection against measles can be derived from distemper virus and have directed our attention during the last 2 years to specific immunization with a measles vaccine. Because of the problem of providing a sufficient antigenic mass for preparation of an effective killed-virus vaccine, we have focused on the evaluation of an attenuated live-virus vaccine. This approach became promising following the adaptation of measles virus to chick embryo tissue culture and its subsequent attenuation by Enders and his co-workers.[9]

STUDIES WITH ATTENUATED LIVE MEASLES VIRUS*

The measles virus used for preparation of the experimental vaccines was the Edmonston strain.† The virus was received in our laboratories as the thirteenth passage in chick embryo tissue culture. Two different vaccines were prepared from it, as shown in Table XIII, and were injected subcutaneously for the clinical studies.

To prepare vaccine A, the virus was passed twice in tissue culture monolayers of chick embryo lung and then 5 times in the amniotic sac of embryonated chicken eggs. The vaccine was an amniotic-sac suspension of the fifth passage and was kept frozen until used, when it was calculated to contain $10^{3.8}$ TCD_{50} per milliliter.

For vaccine B, the original virus was passed 3 times in chick embryo tissue culture, once in monkey kidney monolayer, and finally back into chick embryo tissue culture. The vaccine was a vacuum-dried tissue culture fluid, and titrated $10^{2.0}$ TCD_{50} per milliliter.

Virus assays and antibody titrations by the serum-neutralization method were carried out in HeLa cells.

The first study was instituted to determine the gross and dose response of children to both vaccines. Most of the children were patients in the Pediatric Division of Sea View Hospital, an institution for the care and treatment of tuberculosis. In children selected for the study the disease had been stabilized and was controlled. They were presumed susceptible to measles on the basis of their histories and ages.

Table XIV summarizes the schedule of vaccination and sampling of blood and nasopharynx. Vaccine B was administered to two groups of children, the 6 in Group 1 receiving 1 ml. (100 TCD_{50}) and the 5 in Group 2, 0.5 ml. Vaccine A was administered to the other six groups. The 5 children in Group 3 received 1 ml.; the 5 in Group 4, 0.5 ml.; and the others the following virus concentrations in 1 ml.: Group 5 (4 children), 6,000 TCD_{50}; Group 6 (2 children), 600 TCD_{50}; Group 7 (3 children), 60 TCD_{50}; and Group 8 (3 children), 6 TCD_{50}. Prevaccination blood specimens were taken from all the children and second specimens were taken from Groups 5 through 8 on the twenty-ninth day and from Groups 1 through 4 on the twenty-fifth day. From the latter groups nasopharyngeal swabs also were taken, on the seventh, ninth, and eleventh days.

Pre- and postvaccination antibody titers are given in Table XV. Twenty of the 33 children were serologically negative for measles antibody before vaccination, and all of them responded with antibodies to the

*For the clinical studies reported here, we wish to acknowledge the cooperation of Drs. Joseph Dolgin and Manfred Weichsel,[27] Pediatric Division of Sea View Hospital, Staten Island, N. Y., and Dr. Samuel Karelitz,[28] Head of the Department of Pediatrics, Long Island Jewish Hospital, Long Island, N. Y.

†Obtained from Dr. John F. Enders, Boston, Mass.

Table VIII. Schedule of immunization of ferrets with measles virus

Day	*Inoculum*		*Dose (ml.)*	*No. of injections*	*Route*	*Serum sampled*
	*Virus and adjuvant**	*Virus† only*				
0	×		2.0	2	IM‡	×
		×	1.0	1	IP§	
7		×	1.0	1	IP	
14	×		2.0	2	IM	
		×	1.0	1	IP	
21		×	1.0	1	IP	×
31						×

*Virus-adjuvant mixture: 5 parts virus suspension, 4 parts Bayol F, 1 part Arlacel A.

†Virus suspension: $10^{4.5}$ to $10^{5.6}$ TCD_{50} per milliliter.

‡IM = intramuscular.

§IP = intraperitoneal.

injection of attenuated virus, regardless of the type of vaccine administered, and including even those who received only 6 TCD_{50} of virus.

Table XVI summarized the correlation between antibody response to vaccination and prevaccination antibody level. It should be noted that no booster response was shown by any of the children with prevaccination titers of 1:16 or greater, or by 2 of the 4 children with titers of 1:4 to 1:8.

All of the 11 children who received vaccine B, including 5 seronegatives, remained completely asymptomatic, except for one child who developed a temperature of 103° F. (39.5° C.) on the eighth day after vaccination. Examination of that child revealed acute suppurative tonsillitis. The white blood count was 16,000 per cubic millimeter and the throat culture revealed hemolytic staphylococcus; there were no clinical signs of measles.

Clinical findings in the children who received vaccine A are correlated with their prevaccination antibody status in Table XVII. All but one of the 15 susceptible children (those with prevaccination antibody titers of <1:4) had a febrile reaction lasting 2 to 4 days and ranging from 101 to 103° F. (38.3 to 39.5° C.), with occasional higher temperature recordings. In 8 of these children a faint maculopapular rash was observed, involving the face and, occasionally, the chest, abdomen, and extremities. The eruption disappeared quickly, lasting no more than a day or two. No unequivocal

Table IX. Antibody titers of ferrets immunized with measles or modified canine-distemper virus and their subsequent response to challenge with virulent distemper virus

Group	*Neutralizing antibody titers**						*Mortality ratio following CD challenge*
	MV†			*CD‡*			
	Pre	*21 days post*	*31 days post*	*Pre*	*21 days post*	*31 days post*	
1 (MV immunized)	<4	>60	20	<5	<5	<5	3/3§
2 (MV immunized)	<4	60	10	<5	<5	<5	3/3‖
3 (Control)	<4	<4	<4	<5	<5	<5	3/3§
4 (Control)	<4	<4	<4	<5	<5	<5	3/3‖
5 (CD-immunized)	<4	<4	<4	<5	1,800	1,300	0/2§

*Expressed as reciprocal of serum dilution.

†MV = measles virus.

‡CD = distemper virus.

§Challenge dose = 10,000 LD_{50}.

‖Challenge dose = 1,000 LD_{50}.

Table X. Measles and canine-distemper neutralizing antibody titers of measles patients and their clinical signs

Patient No.	*Clinical signs*	*Antibody titers**			
		Measles		*Distemper*	
		Acute	*Convalescent*	*Acute*	*Convalescent*
1	Koplik spots	<8	44	<5	10
2	Koplik spots	<8	64	<25	5
3	Koplik spots	<8	90	<5	5
4	Koplik spots	<4	20	<5	<5
5	Koplik spots	<8	64	<25	<5
6	Koplik spots	<8	90	<5	<5
7	Koplik spots	<4	128	<25	<5
8	Koplik spots	6	80	<25	<5
9	Rash—2nd day	10	64	<5	5
10	Rash—3rd day	8	8	<5	<5
11	Rash—3rd day	10	64	<5	<5
12	Rash—3rd day	8	32	<5	<5
13	Not obtained	—	40	—	<25
14	Not obtained	—	128	—	<5
15	Not obtained	—	128	—	<5
16	No clinical measles	64	90	10	30
Controls:					
Measles-immune monkey serum			140		20
Distemper-immune dog serum			4		4,000

*Expressed as reciprocal of serum dilution. Serum tested for measles against 100 TCD_{50} of virus, for distemper against 100 to 500 CED_{50} of virus.

Koplik spots were observed, although suspicious spots were noted in one child. All 10 of the children from whom white blood counts were obtained had a leukopenia and a relative lymphocytosis, but none of the 15 children showed any of the catarrhal symptoms associated with natural measles, such as cough, coryza, or conjunctivitis.

None of the children appeared toxic or acutely ill, even when the temperature was significantly elevated; without careful scrutiny many of the symptoms noted could have escaped detection. Thus the clinical picture was similar to that of measles modified by the administration of gamma globulin, but the incubation period was shorter, ranging from 5 to 8 days as judged by the onset of fever or skin eruption, whichever occurred first. No unfavorable effect on the tuberculous process was observed in any of the vaccinated children.

Table XI. Multiplication of measles and canine-distemper viruses in the presence of homologous and heterologous antisera*

Serum added to medium	*Titers†*	
	MV	*CD‡*
None	—	4.2
Normal horse	5.5	—
Normal dog	—	4.2
MV-immune monkey	2.5	4.3
CD-immune dog	5.5	(Negative in undiluted form)

*Measles virus was grown in HeLa cell; distemper virus in chick embryo, tissue cultures.

†Expressed as log 10.

‡EID_{50} per milliliter.

It is significant that no virus was recovered from any of the nasopharyngeal swabs, and no virus was detected in blood specimens taken for viremia studies from 2 susceptible children on the third postvaccination day and from a third child on the fourth day.

Finally, as Table XVIII shows, no correlation could be demonstrated between the appearance of symptoms and the serologic response, on one hand, and the virus dose, on the other. The only possible relationship was a prolonged incubation period at lower virus dosage, extending sometimes to 13 days.

A second study was undertaken chiefly to

extend our experience with vaccine B. This, it will be recalled, is a vacuum-dried chick embryo–tissue culture preparation that elicited antibodies in 5 susceptible children without evoking clinical symptoms.

The 22 children in this study were either private patients or, in some instances, the children of staff members, at Long Island Jewish Hospital in New York. They ranged from 1 to 8 years of age, and were selected as susceptible to measles on the basis of their clinical histories. All received a single sub-

Table XII. Measles and canine-distemper antibody titers of cynomolgus monkeys immunized with measles virus

Serum status (before or No. weeks after immunization)	*Neutralizing antibody titers**					
	Monkey No.					
	1, 2 and 6†		*4*		*5*	
	CD	*MV*	*CD*	*MV*	*CD*	*MV*
Before	<5	<4	<5	<4	<5	<4
1	<5	<4	<5	<4	<5	<4
2	<5	20 to 40	<5	30	<5	30
3	<5	90 to 180	<5	360	10	260
4	<5	90 to 260	<5	360	10	510
5	<5	60 to 260	<5	510	<5	260
6	<5	90 to 180	10	510	<5	180

*Expressed as reciprocal of dilution.

†Monkey No. 3 died of pneumonia on the thirteenth day.

Table XIII. Experimental measles vaccines used in clinical studies

Vaccine A	*Vaccine B*
Enders' 13th passage in chick embryo tissue culture	Enders' 13th passage in chick embryo tissue culture
↓	↓
2 passages in chick embryo lung monolayers	3 passages in chick embryo tissue culture
↓	↓
5 passages in amniotic sac of live chick embryos	1 passage in monkey -kidney monolayer
↓	↓
	1 passage in chick embryo tissue culture
↓	↓
Vaccine kept frozen until used	Vaccine dried from frozen state
Titer: $10^{3.8}TCD_{50}$ per ml.	Titer: $10^{2.0}TCD_{50}$ per ml.

Table XIV. Schedule of vaccination and sampling. Measles Vaccine Study 1

Group No.	*No. of children*	*Vaccine*			*Day*		
			Dose				
		Type	*Volume*	*TCD_{50}*	*Vaccinated*	*Blood sampled*	*Naso-pharynx sampled*
1	6	B	1.0	100	None	0 and 25	7, 9, and 11
2	5	B	0.5	50			
3	5	A	1.0	6,000			
4	5	A	0.5	3,000			
5	4	A	1.0	6,000		0 and 29	None
6	2	A	1.0	600			
7	3	A	1.0	60			
8	3	A	1.0	6			

Table XV. Antibody titers before and after injection of various doses of live attenuated measles virus. Measles Vaccine Study 1

Group No.	*Prevaccination (A) or postvaccination (B)*	*Titers (reciprocal of dilution), by subject No.*					
		1	*2*	*3*	*4*	*5*	*6*
1	A	32	512	<4	<4	708	8
	B	48	1,024	16	44	708	8
2	A	<4	32	89	<4	<4	
	B	22	44	64	89	64	
3	A	128	<4	<4	<4	4	
	B	177	354	256	256	22	
4	A	177	<4	<4	<4	708	
	B	89	128	177	89	512	
5	A	<4	<4	16	8		
	B	16	128	16	8		
6	A	<4	8				
	B	64	256				
7	A	<4	<4	<4			
	B	44	64	27			
8	A	<4	<4	<4			
	B	44	256	22			

cutaneous injection of 1 ml. of the vaccine, containing 100 TCD_{50} of virus, and, thereafter, were observed daily for 2 weeks, any deviation from normal being recorded. Blood samples for antibody determination were taken immediately before vaccination and again 6 weeks later, but no attempt was made to isolate virus.

Testing of prevaccination serum samples showed significant levels of neutralizing antibody in only 2 of the children, both of whom remained normal throughout the observation period. As the range of titers summarized in Table XIX shows, all 18 of the susceptible children from whom postvaccination sera were obtained developed neutralizing antibody titers, and in all but one child they were greater than 1:20. Although that child's titer of 1:8 seems low, as mentioned earlier, even this antibody level afforded protection against experimental measles infection, and in 2 of 3 children, suppressed anamnestic response.

All clinical reactions observed in this study are recorded in Table XX. Figures in the last column denote the number of children presenting the indicated symptom at

Table XVI. Correlation between antibody response to vaccination and prevaccination antibody level. Measles Vaccine Study 1

*Prevaccination antibody titer**	*Children responding with fourfold or greater rise in titer/total children*
<4	20/20
4 to 8	2/4
≥16	0/9

*Reciprocal of dilution.

the time of vaccination, or living with an unvaccinated household contact who was febrile during the observation period. In general, reactions were again similar to those of measles modified by gamma globulin. The children did not complain of discomfort, and their symptoms were so mild as to have escaped detection without careful examination by a qualified pediatrician. Fever was the predominant reaction, occurring in 17 of the 20 susceptible children. Temperatures ranged in 13 cases from 100 to 102° F. (38 to 39° C.) and in 4 cases reached 103° or 104° F. (39.5 to 40° C.). However, as in the first study, fevers lasted only a day or two. Furthermore, at least 9 of the children either were already febrile at vaccination or associated during the observation period with a febrile contact. Reddening of the throat was the next most important symptom, occurring in 13 children, 6 of whom associated with febrile contacts. Unlike the first study, a few children did show slight catarrhal symptoms, such as coryza and conjunctivitis with photophobia. Suggestive rash and Koplik spots were each observed in only 3 children.

The inexplicable fact remains that none of the reactions observed in this study occurred in the children who received the same vaccine in the first study. The only apparent difference between the groups is that the other children were institutionalized tuberculosis patients.

Table XVII. Correlation of clinical findings following administration of vaccine A with prevaccination antibody level. Measles Vaccine Study 1

Clinical findings	*Prevaccination antibody level*	
	<4	≥4
Fever	14/15	0/7
Rash	8/15	0/7
Koplik spots	1?/15	0/7
Leukopenia-lymphocytosis	10/10*	0/7
Catarrhal symptoms	0/15	0/7

*White blood counts were not made for 5 children.

Table XVIII. Correlation of vaccine A virus dose with clinical findings and antibody response. Measles Vaccine Study 1

Virus dose in TCD_{50} per child	*Fever*	*Rash*	*Positive antibody response*
6,000	4/5	2/5	5/5
3,000	3/3	2/3	3/3
600	1/1	1/1	1/1
60	3/3	1/3	3/3
6	3/3	2/3	3/3
Total	14/15	8/15	15/15

Table XIX. Antibody levels of susceptible children following administration of vaccine B. Measles Vaccine Study 2

No. of children	*Antibody titers**
1	8
1	22
4	32
1	44
4	64
3	89
2	128
2	177

*Titers are expressed as reciprocal of dilution.

DISCUSSION

Few would dispute the desirability of an effective method of durable immunization against measles. The possibility of utilizing distemper virus as the immunizing agent because of its suggested relationship to measles virus seems remote, and no encouragement for this approach can be derived from the results of cross-immunologic experiments reported in this communication. As has been the case with other viral diseases, the most likely solution appears to be the use of a specific measles-virus vaccine. Recent progress in cultivating measles virus *in vitro,* in a variety of tissues, makes the prospect for such a vaccine very good indeed.

The limited studies reported here, and similar studies by others, clearly indicate that measles antibodies can be elicited by the injection of attenuated measles virus. There is

Table XX. Clinical reactions of susceptible children following administration of vaccine B. Measles Vaccine Study 2

Clinical reaction	*No. of children*	
	Post-vaccination	*At vaccination or associated with febrile contact*
Fever	17	9
Red throat	13	6
Photophobia	9	3
Coryza	7	2
Conjunctivitis	7	3
Red palate	5	–
Cervical lymph nodes	4	1
Koplik spots	3	1
Rash	3	1
Cough	2	1

reason to believe, also, that even low levels of antibody will protect against natural measles, as they have protected children against experimental infection. The clinical reactions, resembling those of gamma globulin–modified measles, that in most instances follow administration of attenuated virus are open to two interpretations, depending on the point of view. By some they may be considered objectionable and by others as evidence of successful vaccination similar to a vaccinal "take." In any case, before a measles vaccine can be said to be at hand, a number of questions must be answered.

Only a vaccine conferring long-lasting immunity is worth consideration. Will the resistance induced by the attenuated measles-virus vaccine be as durable as that following the natural disease? Such resistance may well be an attribute of a vaccine that provokes some clinical symptoms, but only time can provide the answer.

Another serious consideration is the possibility of postvaccination encephalitis. The fact that neither our studies nor those of Enders and his co-workers[12] demonstrated any viremia after experimental vaccination is encouraging in this respect. Moreover, preliminary results of a small trial now in progress give no indication of altered electroencephalographic patterns in children vaccinated with attenuated measles virus. However, a definite answer concerning encephalitis requires additional experience with the vaccine.

A third pertinent question is whether vaccinated children can transmit the infection to others. Measles is known to be transmitted mainly by droplets expelled in coughing and sneezing, and in neither Enders' studies nor ours was virus demonstrated in nasopharyngeal swabs. Furthermore, no secondary cases of measles have been observed in any vaccination trials, whether in institutionalized groups or in individual families, despite the free association of vaccines with other susceptible children.

SUMMARY

The results of several cross-immunologic experiments in various animal species and in tissue culture failed to confirm the relationship between measles and distemper viruses that has been reported by others, dimming the hope that distemper virus might be used as an effective prophylactic against measles. In contrast, vaccination of children with attenuated measles virus promptly induced measles antibodies, even when the concentration of virus administered was only 6 TCD_{50}.

In most instances, vaccination evoked mild clinical symptoms similar to those in gamma globulin–modified measles, the most prominent of these being fever that ranged from 38° to 40° C. and lasted one or two days. Rash also was recorded in a number of children, but Koplik spots and catarrhal signs were unusual. Affected children did not appear to be toxic or acutely ill; this was the case even in those who had significantly elevated temperatures.

No virus was recovered, either from nasopharyngeal swabs taken on the seventh, ninth, and eleventh days after vaccination or from third-day blood samples, and no cases of secondary measles occurred in either institutionalized children or those living with their families.

Questions about the vaccine still to be

answered concern the duration of the immunity provided, the possibility of postvaccination encephalitis, and the ability of vaccinees to transmit the infection. Answers depend on the gradual and careful extension of trials in susceptible children.

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REFERENCES

1. United States Public Health Service, National Office of Vital Statistics, Morbidity and Mortality Weekly Reports **7:** 1, January 3, 1959.
2. Summary of Four-Year Reports on Health Conditions in the Americas, Pan American Sanitary Bureau Scientific Publication **40:** 32, June, 1958.
3. Enders, J. F., McCarthy, K., Mitus, A., and Cheatham, W. J.: Isolation of Measles Virus at Autopsy in Cases of Giant-Cell Pneumonia Without Rash, New England J. Med. **261:** 875, 1959.
4. Gibbs, F. A., Gibbs, E. L., Carpenter, P. R., and Spies, H. W.: Electroencephalographic Abnormality in "'Uncomplicated" Childhood Diseases, J. A. M. A. **171:** 1050, 1959.
5. Herrmann, C.: Immunization Against Measles, Arch. Pediat. **32:** 503, 1915.
   Active Immunization Against Measles, New York J. Med. **29:** 202, 1929.
6. Shaffer, M. F., Rake, G., Stokes, J., and O'Neil, G. C.: Studies on Measles. II. Experimental Disease in Man and Monkey, J. Immunol. **41:** 241, 1941.
7. Enders, J. F., and Peebles, T. C.: Propagation in Tissue Cultures of Cytopathogenic Agents From Patients With Measles, Proc. Soc. Exper. Biol. & Med. **86:** 277, 1954.
8. Enders, J. F., Peebles, T. C., McCarthy, K., Milovanovic, M., Mitus, A., and Holloway, A.: Measles Virus: A Summary of Experiments Concerned With Isolation, Properties and Behavior, Am. J. Pub. Health **47:** 275, 1957.
9. Enders, J. F., Katz, S. L., and Medearis, D. N.: Recent Advances in Knowledge of the Measles Virus, in Pollard, M., editor: Perspectives in Virology, chap. 7, New York, 1959, John Wiley & Sons, Inc., pp. 103-120.
10. Enders, J. F.: Studies in the Virus of Measles, Connecticut Med. **23:** 561, 1959.
11. Katz, S. L., and Enders, J. F.: Immunization of Children With a Live Attenuated Measles Virus, A. M. A. J. Dis. Child. **98:** 605, 1959.
12. Studies on an Attenuated Measles-Virus Vaccine, New England J. Med. **263:** 153-184, 1960.
    I. Enders, J. F., Katz, S. L., Milovanovic, M. V., and Halloway, A.: Development and Preparation of Vaccine. Technics for Assay of Effects of Vaccination, p. 153.
    II. Katz, S. L., Enders, J. F., and Holloway, A.: Clinical, Virologic and Immunologic Effects of Vaccine in Institutionalized Children, p. 159.
    III. Kempe, C. H., Ott, E. W., St. Vincent, L., and Maisel, J. C.: Clinical and Antigenic Effects of Vaccine in Institutionalized Children, p. 162.
    IV. Black, F. L., and Sheridan, S. R.: Administration of Vaccine by Several Routes, p. 165.
    V. Lepow, M. L., Gray, N., and Robbins, F. C.: Clinical, Antigenic and Prophylactic Effects of Vaccine in Institutionalized and Home-Dwelling Children, p. 170.
    VI. Krugman, S., Giles, J. P., and Jacobs, A. M.: Clinical, Antigenic and Prophylactic Effects of Vaccine in Institutionalized Children, p. 174.
    VII. Haggerty, R. J., Meyer, R. J., Lenihan, E., and Katz, S. L.: Clinical, Antigenic and Prophylactic Effects of Vaccine in Home-Dwelling Children, p. 178.
    VIII. Katz, S. L., Kempe, C. H., Black, F. L., Lepow, M. C., Krugman, S., Haggerty, R. J., and Enders, J. F.: General Summary and Evaluation of the Results of Vaccination, p. 180.
13. Zhdanov, W. M., and Fadeyeva, L. L.: Experimental Materials and Observations of Children Immunized With an Anti-measles Allanto-tissue Vaccine, Med. Dosw. Mikrobiol. **9:** 419, 1957.
14. Adams, J. M., and Imagawa, D. T.: Immunological Relationship Between Measles and Distemper Viruses, Proc. Soc. Exper. Biol. & Med. **96:** 240, 1957.
15. Adams, J. M., Imagawa, D. T., Chadwick, D. L., Gates, E. H., and Siem, R. A.: Relationship of Measles and Distemper, A. M. A. J. Dis. Child. **95:** 601, 1958.
16. Carlstrom, G.: Neutralization of Canine-Distemper Virus by Serum of Patients Convalescent From Measles, Lancet **2:** 344, 1957.
    Comparative Studies on Measles and Distemper Viruses in Suckling Mice, Arch. fdg. Virusf. **8:** 527, 1959.
    Correlation Between Canine Distemper and Measles Virus Neutralizing Capacities in Human Sera, Arch. fdg. Virusf. **8:** 539, 1959.
17. Warren, J., Nadel, M. K., Slater, E., and Millian, S. J.: The Canine Distemper-Measles Complex. I. Immune Response of Dogs to Canine Distemper and Measles Viruses, Am. J. Vet. Res. **21:** 111, 1960.
18. Adams, J. M., Imagawa, D. T., Wright, S. W., and Tartan, G.: Measles Immunization With Live Avian Distemper Virus, Virology **7:** 351, 1959.
19. Cabasso, V. J., Kiser, K. H., and Stebbins, M. R.: Distemper and Measles Viruses. I. Lack of Immunogenic Crossing in Dogs and Chickens, Proc. Soc. Exper. Biol. & Med. **101:** 227, 1959.

20. Cabasso, V. J., Avampato, J. E., Kiser, K. H., and Stebbins, M. R.: Further Evidence of Immunologic Dissimilarity of Distemper and Measles Viruses, Proc. Soc. Exper. Biol. & Med. **104:** 526, 1960.
21. Experimental Vaccination Against Measles, J. A. M. A. **173:** 861-872, 1960.
    I. Schwarz, A. J. F., Boyer, P. A., Zirbel, L. W., and York, C. J.: Tests of Live Measles and Distemper Vaccine in Monkeys and Two Human Volunteers Under Laboratory Conditions, p. 861.
    II. Hoekenga, M. T., Schwarz, A. J. F., Palma, H. C., and Boyer, P. A.: Tests of Live Measles and Live Distemper Vaccine in Human Volunteers During a Measles Epidemic in Panama, p. 868.
22. Cabasso, V. J., and Cox, H. R.: Propagation of Canine Distemper Virus on the Chorioallantoic Membrane of Embryonated Hen Eggs, Proc. Soc. Exper. Biol. & Med. **71:** 246, 1949.
23. Gillespie, J. H., Baker, J. A., Burgher, J., Robson, D., and Gilman, B.: The Immune Response of Dogs to Distemper Virus, Cornell Vet. **48:** 103, 1958.
24. Eagle, H.: The Minimum Vitamin Requirements of the L and HeLa Cells in Tissue Culture; the Production of Specific Vitamin Deficiencies, and Their Cure, J. Exper. Med. **102:** 595, 1955.
25. Morgan, J. F., Morton, H. J., and Parker, R. C.: Nutrition of Animal Cells in Tissue Culture. I. Initial Studies on a Synthetic Medium, Proc. Soc. Exper. Biol. & Med. **73:** 1, 1950.
26. Cabasso, V. J., Kiser, K., and Stebbins, M. R.: Propagation of Canine Distemper Virus in Tissue Culture, Proc. Soc. Exper. Biol. & Med. **100:** 551, 1959.
27. Dolgin, J., Levine, S., Markham, F. S., Cabasso, V., Weichsel, M., Ruegsegger, J. M., and Cox, H. R.: Immunizing Properties of Live Attenuated Measles Virus, J. Pediat. **57:** 36, 1960.
28. Karelitz, Samuel: Personal communication.

Psychomotor seizures in childhood

A clinical study of 120 cases

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FOR ACCURATE diagnosis, adequate investigation, effective therapy, and likely prognosis, the recognition and differentiation of the various types of epileptic seizures are essential. In epilepsy of childhood, the diagnosis of psychomotor seizures is seldom considered. Although there is a large number of electroencephalographic studies concerned with psychomotor epilepsy,[1-4] there are few devoted to a clinical study. In childhood where the electroencephalographic abnormalities associated with psychomotor epilepsy are less specific than those found in adults,[5, 6] the diagnosis of psychomotor epilepsy rests heavily on clinical evaluation. The following report is based on the observations of 120 cases of psychomotor epilepsy in infants and children. Its purpose is to document the common occurrence of such seizures, to define what we consider a highly typical clinical manifestation, and to discuss related neurological and psychological features and medical management.

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DEFINITION, DESCRIPTION, AND DIFFERENTIAL DIAGNOSIS

One may ask, what do we mean by "psychomotor" epilepsy? We have used the term without implying or considering clinical-anatomic correlations. As with epilepsy of other types, the diagnosis is made primarily on clinical grounds. Of greatest value is an accurate, detailed, historical, and chronological documentation of pre-, peri-, and postictal events. In most instances such data will permit easy recognition of seizure types by one intimately acquainted with the clinical manifestations of convulsive states. To the uninitiated, however, a diagnosis of psychomotor epilepsy may necessarily be one of exclusion. A particular seizure may defy exact classification and yet obviously not be a generalized or a local motor seizure, not petit mal, abdominal nor migrainous equivalent, or any other quickly recognized type of seizure. Apart from these better known manifestations of epilepsy there is a group which is broadly classified as psychomotor and which has an equally characteristic pattern of clinical expression.

With allowances made for limitations of children's verbal expression, their symptomatology is remarkably similar to that reported in adults.[1, 7] Common to all is the sudden onset, usually with warning. Older children relate specific auras or vague indescribable feelings; younger children cry out, run or cling to their parents. Then follows a brief episode, usually a matter of only seconds or minutes, of fairly characteristic pattern without loss of consciousness. Certainly consciousness is altered, but not lost as it is in petit mal or grand mal seizures. The patient usually retains his posture. In the vast majority of these patients there is a relatively prolonged postical period—often for hours—of fatigue, drowsiness, or sleep.

On the basis of seizure phenomena, our cases could be divided into five types. Though symptoms or signs were often common to more than one group, a predominant pattern was evident.

Type I. Common to this group was the sudden *arrest of activity,* apathy, limpness, or stiffening "without loss of consciousness." Several had cephalic, epigastric, or precordial auras. None showed unusual motor activity, and none had mastication. Fourteen cases fell into this group, 11.6 per cent.

Type II. The outstanding feature in these patients was their oral and pharyngeal movements, *masticatory activity,* such as smacking, tasting, puckering, chewing, drooling, swallowing, gagging, or choking. The statement, "I'm sick," frequently heralded a seizure. Some clutched or pointed to their throats; other complained of dryness in the throat or seemed thirsty; 2 complained of "bad taste"; 33 cases fell into this group, 27.5 per cent.

Type III. This group comprised those in whom confusion and *automatic purposeless behavior predominated.* There was disorientation, fighting, kicking, running, or crawling. Speech was incoherent or irrelevant. Several "hummed," laughed, or "acted silly." Twenty patients fell into this group, 16.6 per cent. Five also exhibited mastication.

Type IV. Symptoms of this group were chiefly *psychic.* There were complaints of "dreamlike" states and "funny feelings," auditory, olfactory, and visual hallucinations or illusions, and fear or terror. Thirty-six patients belonged to this type, 30 per cent. Four had associated mastication.

Type V. Fourteen children, 11.6 per cent, had paroxysmal "dizziness" or *vertigo.*

Three cases could not be classified in any of the above groups. One had cyclic vomiting, 2 had paroxysmal shivering, and all 3 were controlled by anticonvulsant drugs.

Of the 10 children who were less than 1 year of age at onset of psychomotor epilepsy, 6 exhibited arrest of activity with staring and pallor, followed by sleep, 3 had masticatory attacks, and 1 had nocturnal episodes of terror.

Many of the patients were referred with the mistaken diagnosis of petit mal epilepsy. This common error stems not from a similarity between the two types, but from a lack of differentiation in types of seizure. In the case of psychomotor epilepsy it is more than a question of terminology. Drugs often effective for petit mal epilepsy are of no value in the psychomotor type and may thus entail unjustified risks considering the occasionally serious side effects of oxazolidine therapy. In one instance of mistaken diagnosis we have seen a patient with a near-fatal case of exfoliative dermatitis due to Tridione. The most common single sign or symptom in psychomotor epilepsy in mastication (35 per cent). Staring or arrest of activity without mastication predominated in 11.6 per cent. Whereas both staring and mouth movements are seen in the petit mal type, there is little similarity otherwise. In petit mal seizures there is no aura. The majority of our children with psychomotor attacks had aura or warning. During a psychomotor seizure, consciousness may be altered but is not "lost." If a patient sees, hears, smells, tastes, or feels during a seizure, it is not likely to be the petit mal type. In petit mal epilepsy there are no after effects. Almost all of the children with psychomotor seizures had postictal phenomena, chiefly drowsiness. Color changes, that is, pallor, flushing, and perioral cyanosis, are common in psychomotor

Table I. Total seizures

Type	*No. of cases*	*%*
Psychomotor	120	8.7
Grand mal	402	29.2
"Syncopal"*	67	4.9
Petit mal	60	4.4
Myoclonic (akinetic)	101	7.3
Jacksonian	204	14.8
Febrile	265	19.2
Breath-holding	68	4.9
Headaches	26	1.9
Abdominal pain	19	1.4
Visual	21	1.5
Combinations of above three types	21	1.5
Total	1,374	

*Aura, loss of consciousness with limpness, or tonus without clonus, and with postictal effects.

epilepsy and rare (we have never seen them) in the petit mal type. Petit mal seizures usually occur many times a day; psychomotor disturbances may do so, but usually do not. The majority of untreated patients with petit may epilepsy, 93 per cent in our experience, will have attacks produced by hyperventilation. Only once have we precipitated a non–petit mal seizure (jacksonian) by over breathing.

Differentiation of paroxysmal automatisms and hallucinations from hysteria, fabrication, behavior disorder, or nightmares is most effectively made by painstaking and time-consuming interrogation. The persistence of a highly stereotyped, unchanging, paroxysmal pattern with no apparent relationship to environmental pressures is suggestive of psychomotor epilepsy. Usually a characteristic aura, confusion, mastication, d r o o l i n g, color changes, speech disturbances, or other ictal and postictal phenomena are present to aid in the differentiation.

INCIDENCE

Psychomotor epilepsy is relatively infrequent in the pediatric age group. In a critical review of the subject, Gastaut[1] considered it to be "a disease of older children, of adolescence, and of the first part of adult life." Lennox[7] found that 10 per cent of epileptic patients less than 5 years of age had psychomotor attacks compared with 34 per cent of those over 40 years of age. Five per cent of Livingston's[8] series of 4,158 epileptic children had psychomotor seizures. In an analysis of 1,054 consecutive cases of epilepsy in the convulsive clinic of St. Louis Children's Hospital, 120 had seizures classified as the psychomotor type, an incidence of 11 per cent. Of the 1,054 patients, 238 experienced 2 types of seizures, 38 had 3 types, and 2 patients had 4 types. The frequency distribution of seizure types is shown in Table I. It is notable that psychomotor epilepsy is twice as common as petit mal. Of the 120 patients with psychomotor epilepsy, 54 were girls, 66 were boys; 88 were white, 32 were Negro children.

GENETIC HISTORY OF EPILEPSY

Considering the organic nature of psychomotor epilepsy, one would expect to find a family history of epileptic seizures in a smaller percentage of the patients. In patients with psychomotor seizures and grand mal epilepsy, Lennox[7] found 2.8 per cent of relatives to be epileptic. In contrast, the percentage was twice this in patients with grand and petit mal types of seizures. However, the age distribution of these patients must be considered: Only 29.6 per cent were less than 20 years of age. In a personal communication to Penfield and Erickson,[9] Lennox stated that "when the symptomatic group of epilepsies is subdivided, there is evidence of inheritance of the condition among those whose seizures begin early in life following trauma, while adult epileptics with brain tumors or traumata show no greater incidence of epileptic relatives than do a group of normal control persons." We have found this to be true. In a review of 114 cases of Jacksonian epilepsy in childhood,[10] a family history of epilepsy was found in 28 per cent of the cases. Our findings in children with psychomotor epilepsy further confirm Lennox's observation. In 20 cases there was either no mention of a family history of seizures or such family history was listed as unknown, uncertain, or distant. In

the remaining 100 cases, the family history was positive in 55 cases. In 12 cases, siblings were affected; in 11, parents; in 25, aunts and uncles; in 3, cousins; and in 4, grandparents. If one limits the positive genetic history to parents and siblings, the incidence is 23 per cent.

AGE AT ONSET

The age of the patient at the time of onset of psychomotor seizure is shown in Fig. 1. One patient who reportedly had psychomotor seizures all of her life is not included. Thirty per cent had an age at onset of less than 3 years. Whereas this may seem to be a remarkably early age for psychomotor epilepsy, by contrast, in our series of 1,054 children with epilepsy of all types, 59 per cent of the seizures began in the first 3 years of life. We believe this early age at onset of seizures in children reflects etiological factors such as congenital cerebral defect, birth injury, and genetic predisposition. The unexpected decline in the older age group may represent a decreased rate of admission of adolescent patients to a "babies and children's" hospital.

PATHOGENESIS

Organic central nervous system lesions and prior convulsions. The significance of antecedent brain damage in psychomotor epilepsy is generally accepted. The theory of Earle, Baldwin, and Penfield[11] of temporal lobe herniation through the incisura of the tentorium during obstetric delivery and consequent sclerosis is particularly pertinent. In a study of 157 cases of temporal lobe epilepsy these authors determined such an etiology in 63 per cent. This explanation is especially attractive. It explains how a multitude of diverse pathologic conditions of the brain —anoxia, trauma, hemorrhage, infection, and severe convulsions—can all lead to the same end result of edema of the cerebrum, herniation of the uncus, and sclerosis of the incisura of the tentorium.

Pathologic changes clearly related to brain damage and/or to seizures were evident in 40 of our cases, 33 per cent. Congenital anomalies (hydrocephalus, microcephalus),

Fig. 1. Age at onset of psychomotor epilepsy.

severe brain damage of undetermined etiology as reflected by an I.Q. of less than 50, hereditary diseases (tuberous sclerosis, Sturge-Weber, and von Recklinghausen syndromes), prematurity, birth injury and anoxia constituted a majority of the apparent etiological factors, 26 of 40 cases. The most common other cause was encephalitis, 6 cases: 2 followed infection with measles, 1 followed rabies vaccination, 1 polioencephalitis, and 2 were listed only as viral encephalitis.

Lennox[7] has emphasized the significance of earlier convulsions in the causation of temporal lobe lesions and consequent cerebral dysrhythmia and psychomotor epilepsy. In his series of 414 patients with psychomotor seizures, 63 per cent also had a history of grand mal attacks, the majority of which, 64 per cent, occurred one or more years prior to the onset of psychomotor epilepsy. In contrast, only 3 per cent had petit mal seizures. This suggested a causal relationship between convulsive and psychomotor attacks. Later, Lennox[12] found febrile seizures "especially suspect" in this association. When epilepsy followed febrile seizures of infancy, 17.1 per cent of the children had psychomotor seizures alone compared with an incidence of 6 per cent in a group of 1,900 office patients.

A review of our own data in this area strongly supports Lennox's contention. Sixty-two children, 51.6 per cent, had seizures of other types. In 51 patients, 82.2 per cent, these occurred prior to the onset of psychomotor attacks. The time between earlier

seizures and psychomotor epilepsy ranged from several months to 12 years. In 23 of the 51 cases, the interval exceeded 4 years. The distribution of preceding seizures is as follows: febrile, 27; grand mal, 14; jacksonian, 3; breath-holding, 2; petit mal, 1; myoclonic, 1; visual, 1; cephalic, 1; and abdominal epilepsy, 1. The single case of petit mal seizure is the only one in the series, and so re-emphasizes the rarity of this combination, 0.8 per cent.

At the first visit to our clinic, 263 of 1,054 children with convulsions gave a history of febrile seizures at onset. One hundred and thirty-eight had already experienced nonfebrile seizures. Twenty-seven of these, 19.5 per cent, had psychomotor attacks compared with an 11 per cent incidence in all patients and an 8.7 per cent incidence of seizures in the total group of 1,054 children.

The association of severe or repeated motor convulsions and consequent brain damage is well recognized. Under these circumstances, Penfield and Jasper[13] cite several examples of progressive neurological and cortical defects. In addition to the metabolic disturbances concomitant with the epileptic discharge, they considered the vascular changes of the exposed epileptic cortex observed during and after the discharge as significant in the production of these lesions.

We have seen this course of events in our review of 114 cases of jacksonian epilepsy at St. Louis Children's Hospital.[10] Progressive, ultimately permanent, postepileptiform paralysis developed in at least 10 patients in whom seizures were recurrent or prolonged.

With febrile seizures there is the superimposed harmful effect of the fever itself.[14]

PERSONALITY AND INTELLECTUAL ASPECTS

We have been interested in the psychological development of these children and have referred more than one fourth of the group for psychological testing. Of the 34 children referred, only 29 responded adequately to complete the testing. The tests were administered over a 10-year period by different examiners. It would, therefore, seem probable that the observations on the intellects and personalities of these children would be unbiased.

The group tested consisted of 17 boys and 12 girls. Their ages ranged from 4 to 15 years. Five were Negro and 24 were white children.

The I.Q. range for these 29 children was from a low score of 34 to a high of 116. Approximately one half were considered low average or above in intellectual functioning (I.Q. rating of 80 or more). The remainder scored downward from borderline to severe mental deficiency. Fourteen untested children were also observed to be mentally deficient and were so noted in their charts. Only 24 per cent of the tested group scored an I.Q. of 90 or above. Considering the fact that 77 per cent[15] of the general population scores an I.Q. of 90 or above, this does not speak well for our group. Twenty-four per cent were considered low average (I.Q. of 80 to 89) as compared with 15 per cent in the general population. Twenty-eight per cent were of borderline deficiency (I.Q. of 70 to 79), and 24 per cent were mentally deficient (I.Q. of 69 or below). Since only about 8 per cent of all individuals fall within the two latter categories, the percentage of mental deficiency in the group of tested children is more than six times that seen in the general population. If we include the 14 intellectually deficient children who were not tested, close to one fourth of the total series were deficient. It should be noted, however, that the examiners indicated that more than half of the tested children showed signs of organic brain damage which may have been a contributing factor to their low intellectual functioning.

Since there is considerable interest in the personality make-up of all epileptics, we are reporting a summary of the personality test findings on this group of children. These findings are not to be considered universally applicable, nor do they necessarily characterize the entire group of children included in this series.

As mentioned above, more than half of the tested group displayed the type of be-

havior frequently associated with organic brain damage. Among the features noted were hyperactivity, excitability, poor memory, poor visual-motor coordination, and short attention span. Many of them were described as anxious and fearful with tendencies toward being impulsive and poorly controlled. There was a trend on the part of parents to be overprotective. The majority showed relatively poor social development as might be anticipated when one considers their intellectual development. Three children who were having difficulties in meeting their environmental situations because of emotional problems were considered capable of higher intellectual functioning. The majority were thought to be insecure, and a number hostile. On the whole, the behavior problems exhibited were similar to those seen in any child guidance clinic.

ELECTROENCEPHALOGRAPHY

The origin of epileptic discharge in this group of patients is problematic. Some, undeniably, have temporal lobe auras and, on a clinical-anatomic basis, would perhaps best be classified as having temporal lobe seizures. Others deny the purely temporal lobe origin of psychomotor seizures. The controversy over classification and the association of these seizures with EEG foci are reviewed in great detail by Gastaut.[1] Electroencephalograms were obtained in 117 of the 120 cases. Seventeen tracings were indeterminate. The remaining 100 were reported as follows:

Resting record. In 26, the frequencies during the resting period showed slow dysrhythmias; in 2, the frequency was fast; in 16, it was mixed fast and slow. Paroxysmal activity was evident in 48 cases: 40 slow, 1 fast, 7 mixed. Six tracings were read as definite spike-wave dysrhythmia. In 2 cases the frequencies were normal but showed intermittent nonfocal spiking.

Focal signs. Focal abnormalities were seen in 28 of the records: 13 showed slow focal patterns (2 frontal, 3 central, 4 temporal, 3 occipital, and 1 generalized but unilateral); 8 records showed focal spiking (6 temporal, 2 central); 4 records had spike-wave foci (1 temporal, 1 central, and 2 occipital); 3 patients had slow focal trends (1 central, 1 anterior, and 1 posterior).

Amplitude asymmetry. One record showed an amplitude asymmetry (temporal).

MEDICAL TREATMENT

For the purpose of assessing the results of treatment, the cases were divided into those with complete control, those not completely controlled, and those in which the period of observation was inadequate for evaluation (less than 6 months).

Forty-one cases, one third of the total, were 100 per cent controlled, 40 were not completely controlled, and the remainder had only limited follow-up.

From the reports of medical treatment of psychomotor epilepsy, Gastaut[1] concluded that diphenylhydantoin had no effect and barbiturates "hardly any" on psychomotor attacks. Several discussants of Gastaut's paper[1] disagreed with respect to the response to Dilantin. We agree with them and extend this difference of opinion to include the barbiturates.

Our patients are started on phenobarbital alone. The dosage range is from 15 to 90 mg. at bedtime. This dose is seldom exceeded despite the absence of side effects. If phenobarbital fails to achieve complete seizure control, Dilantin is added. Most children can tolerate 4 mg. per kilogram of body weight per day in one or divided doses. Increments are made as tolerated, if required. If seizures persist, Mysoline is added to previous therapy in increasing dosage up to 8 to 12 mg. per kilogram of body weight per day (3 divided doses). If the serious risks are respected and the necessary precautions are taken, both Mesantoin and Phenurone have proved to be of value in refractory cases. Our experience with Celontin in psychomotor epilepsy is limited.

To this list of reputedly effective drugs we would like to add acetazolamide. This drug should be used as an adjuvant and not as a substitute for previous therapy. In our evaluation of Diamox[16] in the treatment of epilepsy in 56 children, there were 11 cases of

intractable "temporal lobe" or psychomotor epilepsy. Six were completely controlled. In the remaining 5, the addition of Diamox was of little or no value.

Most authorities agree that surgical therapy should be reserved for those patients who fail to respond to an adequate trial of anticonvulsant medicine. With the drugs presently available, a thorough evaluation of medical therapy requires months and sometimes several years. In the few cases referred for surgical consideration, determination of localization by clinical, laboratory, and pneumoencephalographic examinations has presented a major difficulty.

SUMMARY

One hundred and twenty cases of psychomotor epilepsy in children have been reviewed.

1. Eleven per cent of 1,054 pediatric patients with epilepsy had psychomotor attacks. In our experience psychomotor epilepsy is twice as common as the petit mal type.

2. Clinically, the cases could be divided broadly into 5 types of behavior: arrest of activity, masticatory, automatic, psychic, and vertiginous.

3. Fifty-five children had a family history of convulsions; parents or siblings were affected in 23.

4. Thirty per cent had an age at onset of 3 years or less. This is half the percentage of that seen in 1,054 cases of all types of epilepsy in childhood.

5. One third of the children had an obvious background of organicity. The etiological significance of previous convulsions, particularly febrile seizures, is emphasized.

6. The majority of cases were misdiagnosed as petit mal seizures. Hysteria, behavior disorder, nightmares, and fabrication must also be ruled out.

7. One fourth of those in the series were mentally deficient children as determined clinically or by psychomotor testing. One half of those in the tested group exhibited behavior disorders.

8. Of 117 electroencephalograms, only 17 were normal or indeterminate.

9. About one third of the patients with psychomotor epilepsy were completely controlled, another third were not controlled, and one third were inadequately followed for proper evaluation. Acetazolamide as an adjuvant to previous therapy is recommended in intractable cases.

REFERENCES

1. Gastaut, H.: So-called "Psychomotor" and "Temporal" Epilepsy, Epilepsia **2:** 59, 1953.
2. Gibbs, F. A., and Gibbs, E. L.: Atlas of Electroencephalography, Vol. II, Epilepsy, Cambridge, Mass., 1952, Addison-Wesley Press, pp. 162-171.
3. Hill, D.: The Electroencephalographic Concept of Psychomotor Epilepsy: A Summary, IV Congrès Neurol. International **1:** 27, 1949.
4. Merlis, J. K., Grossman, C., and Henriksen, G. F.: Comparative Effectiveness of Sleep and Metrazol-Activated Electroencephalography, Electroencephalog. & Clin. Neurophysiol. **3:** 71, 1951.
5. Glaser, G. H., and Dixon, M. S.: Psychomotor Seizures in Childhood; Clinical Study, Neurology **6:** 646, 1956.
6. Glaser, G. H., and Golub, L. M.: Electroencephalogram of Psychomotor Seizures in Childhood, Electroencephalog. & Clin. Neurophysiol. **7:** 329, 1955.
7. Lennox, W. G.: Phenomena and Correlates of Psychomotor Triad, Neurology **1:** 357, 1951.
8. Livingston, S.: Diagnosis and Treatment of Convulsive Disorders in Children, Springfield, Ill., 1954, Charles C Thomas, Publisher.
9. Penfield, W., and Erickson, T. C.: Epilepsy and Cerebral Localization: A Study of the Mechanism, Treatment and Prevention of Epileptic Seizures, Springfield, Ill., 1941, Charles C Thomas, Publisher.
10. Holowach, J., Thurston, D. L., and O'Leary, J.: Jacksonian Seizures in Infancy and Childhood, J. PEDIAT. **52:** 670, 1958.
11. Earle, K. M., Baldwin, M., and Penfield, W.: Incisural Sclerosis and Temporal Lobe Seizures Produced by Hippocampal Herniation at Birth, A. M. A. Arch. Neurol. & Psychiat. **69:** 27, 1953.
12. Lennox, W. G.: Significance of Febrile Convulsions, Pediatrics **11:** 341, 1953.
13. Penfield, W., and Jasper, H.: Epilepsy and the Functional Anatomy of the Human Brain, Boston, 1954, Little, Brown & Company.
14. Lennox, M. A.: Febrile Convulsions in Childhood: Their Relationship to Adult Epilepsy, J. PEDIAT. **35:** 427, 1949.

15. Terman, L. M., and Merrill, M. A.: Stanford-Binet Intelligence Scale (Manual for the Third Revision, Form L-M), Boston, 1960, Houghton Mifflin Company.

16. Holowach, J., and Thurston, D. L.: A Clinical Evaluation of Acetazolamide (Diamox) in the Treatment of Epilepsy in Children, J. PEDIAT. 53: 160, 1958.

Allergenicity of cow's milk proteins

IV. Relationship to goat's milk proteins as studied by serum-agar precipitation

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THE status of milk substitutes for infants who are sensitive to cow's milk has recently been reviewed by Dees.[1] Most textbooks of allergy and pediatrics state that, at times, goat's milk may be used as a substitute for cow's milk in milk hypersensitivity.[2-6] The reason given for this statement is that the whey proteins of goat's and cow's milk are thought to be immunologically species specific. This can be traced to the misinterpretation of experiments by Versell[7] in 1915 on the antigenic relationship of proteins of various species of animals, when the complement fixation technique is used. This worker studied the relationship of cow's whey protein to goat's and human's lactalbumin. He found that the bovine whey reacted with goat's milk whey, but to a slightly lesser degree than with cow's milk lactalbumin. Versell did find, however, that bovine whey and human whey proteins were species specific.

In 1939 Hill[8] reported on 45 eczematous infants who gave positive intracutaneous reactions to cow's lactalbumin. These infants were then skin tested intracutaneously with goat's lactalbumin. Ten gave doubtful reactions and 25 gave positive dermal reactions to goat's lactalbumin. At that time, Hill concluded that cow's and goat's lactalbumin were by no means entirely species specific.

Saperstein,[9] using precipitin tests, has recently demonstrated that antisera to bovine alpha lactalbumin and beta lactoglobulin reacted with the proteins of the goat's milk whey.

Alpha casein, alpha lactalbumin and beta lactoglobulin are the most important fractions in cow's milk and make up approximately 73 per cent of the total milk proteins.

The purpose of the experiments reported herein was to study the antigenic relationship of goat's and cow's milk proteins by the double-gel-diffusion method of Ouchterlony.[10] Gel-diffusion immunologic techniques are essentially antigen-antibody precipitin reactions in a semisolid medium. The double-gel-diffusion technique has been found to be a useful method for studying the qualitative immunologic relationship. By this technique, direct comparison can be made between several antigenic substances or antisera.

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Fig. 1. *A,* Raw cow's milk; *H,* raw goat's milk; *j,* α-casein antisera.

MATERIALS AND METHODS

The whole milks studied were raw and homogenized cow's milk, and raw goat's milk. These were obtained from local commercial sources.

The bovine alpha casein, beta lactoglobulin and alpha lactalbumin were the same as those used in a previous paper.[11]

Rabbit antisera were prepared against whole, undiluted raw and homogenized cow's milk and against whole, undiluted raw goat's milk as well as against bovine alpha casein, beta lactoglobulin, and alpha lactalbumin fractions. The bovine fractions were prepared in a dilution of 1 to 10. The above antigens were emulsified with equal volumes of Freund's complete adjuvant. Rabbits were injected subcutaneously 3 times a week for 4 weeks. A week later 20 to 30 ml. of blood was taken from the animals by cardiac puncture. The antisera all gave a positive precipitin reaction when the tube precipitin test was used.

Petri dishes were coated with a layer of 1.3 per cent agar and Veronal buffer (pH 8.6) containing thimerosal (Merthiolate) as a preservative. After the agar gelled, small wells were punched in the agar, spaced 15 to 20 mm. apart, and 0.02 to 0.10 ml. of the various whole milks or fractions was placed in 2 of these wells and a similar quantity of antisera was placed in the remaining well. Diffusion of the milk antigens and rabbit antisera occurred in the agar, and bands of precipitation were observed.

The resulting precipitation bands and the zone of equivalence produced by the diffusion of the milk antigens and antisera were recorded by free-hand drawings, and by color or black and white photographs at 24-hour intervals.

RESULTS

Fig. 1 shows the results obtained when bovine alpha casein antiserum diffused against raw cow's milk and raw goat's milk. A common precipitin band forms a pattern of identity. The band forming against the raw goat's milk is less dense.

Fig. 2 shows an Ouchterlony plate in which bands of identity were obtained when raw cow's milk and raw goat's milk were diffused against bovine antilactoglobulin. The precipitation bands of identity suggest that, immunologically, bovine lactoglobulin and goat's lactoglobulin are not species specific.

Results obtained when raw cow's milk and raw goat's milk were diffused against bovine alpha lactalbumin antisera are shown in Fig. 3. Two precipitin bands can be seen from the interaction of raw cow's milk and bovine alpha lactalbumin. One distinct precipitin line can be seen between raw goat's milk and bovine lactalbumin antisera. This band forms a line of identity with one of the bands of the raw cow's milk–antilactalbumin

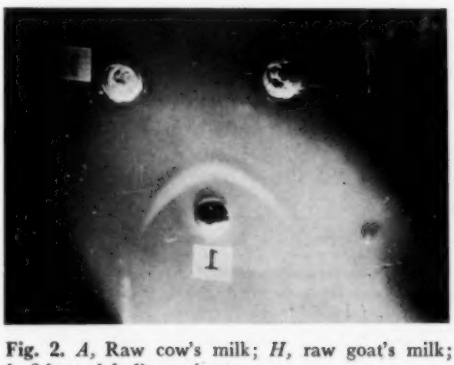

Fig. 2. *A,* Raw cow's milk; *H,* raw goat's milk; *l,* β-lactoglobulin antisera.

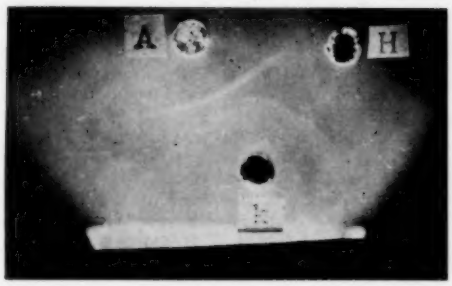

Fig. 3. *A*, Raw cow's milk; *H*, raw goat's milk; *k*, α-lactalbumin antisera.

reaction. This would suggest that a major antigen is common to both and that bovine lactalbumin is immunologically related to the lactalbumin in goat's milk.

Although these protein fractions in the milk have been shown by electrophoresis to be highly pure, the presence of the 2 precipitation bands between raw whole milk and the beta lactoglobulin and alpha lactalbumin antisera indicates some contamination. The contaminant was found to be an antigen identical with bovine serum albumin.[12] Other workers[13, 14] have shown that bovine serum albumin is identical with an albumin fraction that has been crystallized from milk.

DISCUSSION

The presence of a common antigen in the casein fraction of cow's milk and goat's milk has been known for some time. However, it has been generally accepted that bovine and goat's lactoglobulin and lactalbumin are species specific.

The gel-diffusion plates clearly show that precipitation bands formed by raw cow's milk and raw goat's milk diffusing against bovine beta lactoglobulin and alpha lactalbumin antisera formed bands of identity. This joining of the precipitation bands forming symmetrical precipitation lines indicates a common antigen. Our findings indicate that goat's lactoglobulin and lactalbumin are immunologically related to bovine lactoglobulin and lactalbumin.

Our data on the immunologic relationship of cow's and goat's milk proteins suggest that raw goat's milk would not be indicated as a milk substitute in cow's milk hypersensitivity.

SUMMARY

Double-gel-diffusion precipitation analysis of the relationship between cow's and goat's milk reveals:

1. Cow's milk and goat's milk casein have precipitin patterns of almost complete identity with each other. This tends to confirm previous opinions that goat's casein is closely related to cow's casein, antigenically.

2. Although it had been generally accepted that the whey proteins of the goat and cow are species specific, our precipitation-in-gel experiments indicate that goat's lactoglobulin and lactalbumin are immunologically related to bovine lactoglobulin and lactalbumin.

3. Our experiments cast doubt on the value of substituting raw goat's milk for cow's milk preparations in the treatment of infants and children allergic to cow's milk.

We wish to express our appreciation to Dr. James N. Etteldorf for his interest and advice in the preparation of this manuscript.

REFERENCES

1. Dees, S. C.: Allergy to Cow's Milk, Pediat. Clin. North America **6**: 881, 1959.
2. Cooke, R. A.: Allergy in Theory and Practice, Philadelphia, 1947, W. B. Saunders Company.
3. Nelson, W. E.: Textbook of Pediatrics, ed. 7, Philadelphia, 1959, W. B. Saunders Company.
4. Holt, L. E., and McIntosh, R.: Pediatrics, ed. 12, New York, 1953, Appleton-Century-Crofts, Inc.
5. Sherman, W. B., and Kessler, W. R.: Allergy in Pediatric Practice, St. Louis, 1957, The C. V. Mosby Company.
6. Sheldon, J. M., Lovell, R. G., and Mathews, K. P.: A Manual of Clinical Allergy, Philadelphia, 1953, W. B. Saunders Company.
7. Versell, A.: Ueber das serologische Verhalten von Milch und Milch-Eiweisskorpern in frischen und gekochten Zustande, Ztschr. Immunitätsforsch. **24**: 267, 1915.

8. Hill, L. W.: Immunologic Relationship Between Cow's Milk and Goat's Milk, J. PEDIAT. **15:** 157, 1939.
9. Saperstein, S.: Antigenicity of the Whey Proteins in Evaporated Cow's Milk and Whole Milk, Ann. Allergy **18:** 765, 1960.
10. Ouchterlony, O.: Antigen-Antibody Reactions in Gel. IV. Types of Reaction in Coordinated Systems of Diffusion, Acta path. et microbiol. scandinav. **32:** 231, 1953.
11. Crawford, L. V.: Allergenicity of Cow's Milk Proteins. I. Effect of Heat Treatment on the Allergenicity of Protein Fractions of Milk as Studied by the Dual Ingestion Passive Transfer Test, Pediatrics **25:** 432, 1960.
12. Crawford, L. V., and Grogan, F. T.: Allergenicity of Cow's Milk Proteins. II. As Studied by Serum-agar Precipitation, Pediatrics (in press).
13. Polis, B. D., Shmulkar, H. W., and Custer, J. H.: Isolation of a Crystalline Albumin From Milk, J. Biol. Chem. **187:** 349, 1950.
14. Coulson, E. J., and Stevens, H.: The Serological Relationship of Bovine Whey Albumin to Serum Albumin, J. Biol. Chem. **187:** 355, 1950.

Circumscribed intraventricular hematoma in infants

Associated with craniosynostosis and secondary hydrocephalus

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IN GENERAL, hemorrhage into the ventricles of the brain may be caused by bleeding from trauma or from adjacent aneurysms, angiomas, or neoplasms. When present in small or even moderate quantities, the blood mixes homogeneously with ventricular fluid and is eventually absorbed if the patient survives; clotting does not take place because of the inhibiting influence of the ventricular fluid. When present in such quantity as to be associated with clotting, an intraventricular hemorrhage is usually associated with such a massive episode of bleeding that death of the patient ensues.

Only rarely do discrete hematomas form in the ventricles. When they do, signs and symptoms develop which may be confused with those of an intraventricular neoplasm. There is little mention of this phenomenon in the literature. Two previous reports have appeared describing circumscribed intraventricular hematomas. Avol and Vogel[1] in 1955 reported 2 cases of circumscribed hematoma in the lateral ventricle which simulated an encapsulated neoplasm. The lesions were removed successfully but no specific etiology was determined. These patients were 48 and 14 years of age, respectively. Caram, Sharkey, and Alvord[2] more recently described a patient 34 years old in whom a hematoma in the lateral ventricle due to slow hemorrhage from a thalamic angioma was associated with a berry aneurysm of the anterior choroidal artery.

Intraventricular hemorrhage is a common cause of death in the premature newborn infant. Rohrbach,[3] Arey,[4] and other investigators, in reviewing autopsies on fetuses and infants, have noted hemorrhage into the ventricles to be a surprisingly frequent finding, apparently unassociated with vascular anomalies, neoplasms, or objective evidence of associated trauma. This finding is much less frequent in mature newborn infants than in small premature ones and in fetuses.

Circumscribed hematoma of the third ventricle in a mature infant and its successful removal has not, to our knowledge, heretofore been reported. Reference will be made to an additional infant with a discrete hematoma of the lateral ventricle, and the clinical implications of such hemorrhages will be discussed.

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Fig. 1. Case 1. Pneumoencephalogram. Note enlargement of the lateral and third ventricles and normal aqueduct of Sylvius and fourth ventricle. The shadow of the hematoma can be seen in the posterior superior third ventricle.

CASE REPORTS

Case 1. L. T., a 3-week-old white male infant was admitted to St. Christopher's Hospital for Children on Oct. 3, 1959, because of a convulsive episode followed by the development of spasticity of the neck and extremities. Birth had been at term, requiring forceps rotation and extraction. There was said to have been a 10-minute period of apnea which necessitated resuscitation.

Examination on admission revealed a well-nourished, irritable infant. The anterior fontanel was small but open and flat. The skull was dolichocephalic in its configuration and measured 14½ inches in circumference; the chest measured 13½ inches. The pupils were equal and reacted to light. There was an occasional nystagmus to the left. No papilledema was present. The cranial nerves, as far as could be tested, were functioning normally. There was conspicuous nuchal rigidity, and the infant was opisthotonic with severe spasticity of all four extremities, adductor spasm of the lower extremities, bilaterally positive Babinski's signs, and generalized hyperreflexia.

Bilateral subdural taps yielded small quantities of xanthochromic fluid. A lumbar puncture disclosed bloody spinal fluid under moderately increased pressure. Plain x-rays of the skull revealed evidence of increased intracranial pressure as manifested by widening of the coronal and lambdoidal sutures. There was craniosynostosis of the sagittal suture. The infant was treated conservatively for the next 21 days with improvement of his general condition, but spasticity of the extremities, hyperreflexia, and nuchal rigidity persisted. A repeat lumbar puncture on the twenty-first day after admission revealed clear, xanthochromic fluid with a slightly elevated pressure. At this time, the head measured 15¾ inches, an increase of 1¼ inch in 3 weeks. Pneumoencephalography now disclosed mild ventricular dilatation and the presence of a mass in the posterior superior portion of the third ventricle (Fig. 1).

Bilateral parasagittal craniectomies were performed on Nov. 3, 1959, to correct the craniosynostosis. A hematoma was completely removed from the third ventricle, following which the inside of the ventricle was found to be normal except for discoloration. Clear xanthochromic fluid welled into and through the ventricle. There was no evidence of angioma or of any vascular anomaly.

Histologic examination of the mass removed demonstrated only blood clot and no evidence of additional tissue.

The infant's postoperative course was uneventful. Signs of increased intracranial pressure subsided and the spasticity was markedly improved during the 3-week period following the craniotomy. At the end of 3 months, he exhibited no objective neurological signs except for a mild alternating strabismus. He seemed to be growing and developing normally.

At the time of writing, he is 17 months of age and is reported to be growing and developing at a normal rate. There is some delay in walking, but neurological examination fails to demonstrate a deficit in the legs which will prohibit walking.

Case 2. G. G., a 3½-week-old white male infant was admitted to St. Christopher's Hospital for Children on Oct. 27, 1955, because

of an enlarging head, convulsive seizures, and failure to thrive. Delivery had been difficult because of a large head, requiring forceps rotation and extraction. A cephalhematoma had been present and an abrasion of the right ear oozed for 36 hours after birth. Two weeks after birth a spinal tap yielded bloody fluid. A single generalized convulsion had occurred the day prior to admission.

On examination, the infant had an obviously enlarged head which measured 15¾ inches. The suture lines were widely separated and the anterior fontanel was tense and bulging. His general appearance was otherwise normal and he had a good cry. Several generalized seizures occurred on the day of admission, and these were controlled with phenobarbital. A lumbar puncture yielded bloody, xanthochromic fluid. Bilateral subdural taps were negative. A ventricular tap yielded dark brown xanthochromic fluid. Ventriculography demonstrated a mass lying in the right lateral ventricle at the junction of the body and the temporal horn (Fig. 2). This was considered to be a papilloma of the choroid plexus. There was symmetrical dilatation of the ventricular system, including the third and the fourth ventricles. Air could not be made to flow into the spinal canal or into the subarachnoid space over the surface of the brain.

Craniotomy was performed and a dark, cauliflower-like mass, approximately 2 by 3 cm., was found attached to the choroid plexus by a thin pedicle of two or three vessels. The mass was removed totally by clipping and sectioning the stalk.

Histologic examination of the mass revealed only choroid plexus and clotted blood with no evidence of neoplasm.

Postoperatively, the infant did well for 3 days, then began to vomit, and the fontanel became bulging. Exploration of the posterior fossa demonstrated dense arachnoiditis with adhesions around the foramina of Magendie and Luschka. Progressive hydrocephalus was controlled by a ventriculocisternostomy and a Matson procedure. He was then discharged improved.

Fig. 2. Case 2. Ventriculogram. Shadow of hematoma can be seen in lateral ventricle.

He was readmitted 6 months later because of increased intracranial pressure and progressive enlargement of the head. The child suddenly died the morning following admission. Autopsy revealed no specific cause of death. There was marked symmetrical dilatation of all ventricles of the brain. There was extensive arachnoid fibrosis in the posterior fossa and around the base of the brain, occluding the outlets from the fourth ventricle. There were no additional lesions within the ventricles. A silver hemostatic clip was found lying across the amputated vessels of the choroid plexus at the site of previous operation.

DISCUSSION

It is well known that intracranial hemorrhage of all types is a principal cause of death in the neonatal period, estimated to range from 30 to as high as 60 per cent of all early deaths. The incidence is especially high in premature infants. In view of this, one must reflect upon the possible causes. The first thought is that they are predominantly traumatic in origin and are secondary to tentorial tears or stresses occurring within the cranium during delivery. However, in a series of 402 autopsies of fetuses, premature infants, and mature liveborn infants, Rohrbach[3] observed only 11 instances (2.7 per

cent) of tentorial tears. Intraventricular hemorrhage, on the contrary, was present in 17.6 per cent of 250 fetuses 20 to 34 cm. in length and in 16 per cent of 100 premature liveborn infants. In 34, or 56.6 per cent, of these 60 fetuses and premature infants, intraventricular hemorrhage was the only significant pathologic finding. None of these hemorrhages were associated with hemorrhages in the skin, parenchyma, or serous membranes, nor were there any mechanical injuries to the outside of the head or other pathologic changes. Such hemorrhages were not found in any of the 52 mature newborn infants. Arey[4] has found small intraventricular bleeding to be concentrated around the engorged vessels of the choroid plexus, whereas the larger hemorrhages arise about the vena terminalis in the subependymal layer between the caudate nucleus and thalamus. From this zone, the blood may subsequently break into the ventricles through the ependyma. Rohrbach also has called attention to this highly cellular subependymal zone in the premature infant as a frequent site of bleeding. This tissue normally regresses at the end of fetal development and matures into nervous and glial elements. The actual cause of bleeding into this zone is unknown, but several factors seem to be significant. Prematurity seems to be one of the principal predisposing factors with its associated deficient development of the elastic fibers in the vascular system, increased capillary fragility, and the frequency of anoxia. Such anoxia may be secondary to intrauterine asphyxia, to venostasis due to uninterrupted labor, compression of the umbilical cord, or placenta previa.

Deficiencies of vitamin C and vitamin K, along with certain blood dyscrasias, must be considered as possible factors in a few instances. However, the large number of infants in whom intraventricular hemorrhage is present without evidence of hemorrhage elsewhere in the body tends to militate against this as a factor of importance in the vast majority of cases of intraventricular hemorrhage in the newborn.

Analysis of these 2 cases in the light of the above facts reveals several factors which seem to be significant. Both infants were born at full term and were delivered with some difficulty by forceps extraction. The first patient had a period of anoxia of several minutes' duration before respiration was established. In the second case, dystocia was caused by a large head, suggesting that the pathologic process, with its associated hydrocephalus, may have begun in utero. In both instances, there was a short period of a week or two in which the infant did not appear to be ill enough for hospitalization. In neither instance was the devastating clinical picture of rapidly developing increased intracranial pressure, pin-point pupils, marked rigidity, unconsciousness, and rapid death apparent, as is so commonly observed in association with intraventricular hemorrhage in the newborn infant. Rather, in both infants, the clinical signs and symptoms were gradual in onset, consistent with bleeding in small amounts under low pressure. It may well be that the maturity of both infants is responsible for their greater durability and ability to survive the acute hemorrhages and thus allow the formation of a circumscribed hematoma. In both instances, the hematoma was in the vicinity of the choroid plexus, suggesting that the hematoma developed as the result of a slow ooze from the vessels of the choroid plexus, with the clot then forming over the bleeding point.

Hydrocephalus was present in some degree in both cases. The cerebrospinal fluid was persistently xanthochromic, perhaps lending some support to the idea that disorders of the choroid plexus which modify it elaborate large quantities of fluid with a high content of protein and might be responsible for some forms of hydrocephalus. This might be said of the first infant who improved after the hematoma in the third ventricle was removed. In the second case, however, extensive subarachnoid fibrosis around the base of the brain, which was probably caused by the same hemorrhage, seemed to be the primary cause of hydrocephalus on a mechanical basis. Nevertheless, the presence of the hematoma on the choroid plexus could

account for the persistence of the dark, xanthochromic fluid within the ventricle.

Any relationship between the hematoma in the third ventricle and craniosynostosis of the sagittal suture line in the first patient is not known.

We were impressed by the spastic quadriplegia which was present in the first patient preoperatively, and even more so by its dramatic improvement following removal of the hematoma from the third ventricle. One cannot speculate accurately how this child would have fared without the removal of the hematoma. It is safe to assume that, having recovered from the acute effects of the hemorrhage, he would have survived with a serious neurological deficit and been classified as having severe cerebral palsy. The detection and removal of the circumscribed hematoma from the third ventricle, however, resulted in alleviation of the spasticity and recovery of motor function. Our chief concern with cerebral palsy is to detect and treat the cause as early as is possible. It is urged, therefore, that all infants who have exhibited evidence of intracranial bleeding and survive, especially those with residual neurological deficit, should have a thorough investigation with contrast studies to detect, if possible, a surgically correctable cause. Ordinarily, in the presence of intracranial hemorrhage, one would consider cerebral angiography as the procedure of choice. However, we doubt that the intraventricular hematomas present in these children would have been detected by this method with our present technique.

SUMMARY

A case of discrete hematoma of the third ventricle in a full-term infant, associated with craniosynostosis and secondary hydrocephalus, is reported. The hematoma was successfully removed and the hydrocephalus subsided. To our knowledge, no such case has been previously reported.

The differential diagnosis and possible etiological factors of intraventricular hemorrhage in infants are discussed. The use of definitive diagnostic studies by contrast methods is urged in selected patients. Only by this means will lesions which may be responsible for a few instances of cerebral palsy be detected at an early stage when curative procedures are still possible.

REFERENCES

1. Avol, Mitton, and Vogel, Philip: Circumscribed Intraventricular Hematoma Simulating an Encapsulated Neoplasm, Bull. Los Angeles Neurol. Soc. **20:** 25, 1955.
2. Caram, P. C., Sharkey, P. C., and Alvord, E. C.: Thalamic Angioma and Aneurysm of the Anterior Choroid Artery With Intraventricular Hematoma, J. Neurosurg. **17:** 347, 1960.
3. Rohrbach, H.: Gehirnventrikelblutungen als häufiger Sektionsbefund bei Feten und Frühgeburten, Zentralbl. Gynäk. **75:** 1709, 1953.
4. Arey, James B.: In Greenhill, J. P., editor: Obstetrics, ed. 12, Philadelphia, 1960, W. B. Saunders Company, pp. 841-842.

An evaluation of early esophagoscopy and corticosteroid therapy in the management of corrosive injury of the esophagus

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WHEN an individual presents with a history of ingestion of a corrosive agent, two problems arise: first, that of ascertaining the presence or absence of burns in the esophagus; second, that of management and treatment.

In this report we wish to present our experience with 76 children who had a history of ingestion of a corrosive agent. These children were admitted to Detroit Receiving Hospital between March, 1957, and April, 1959. The presence or absence of esophageal burns was established by diagnostic esophagoscopy within 24 hours of admission. If esophageal burns were present, corticosteroids were utilized in treatment.

METHOD OF STUDY

In each instance in which a child had contact with a corrosive agent, a presumptive diagnosis of corrosive burns of the esophagus was made, and the child was admitted to the Pediatric Service. Agents suspected of causing corrosive burns were lye, Drano, Lysol, ammonia, Clorox, Clinitest tablets, and potassium permanganate tablets (Table I).

With the patient under general anesthesia, esophagoscopy was performed by the Otolaryngology Service within 24 hours from the time of ingestion. The esophagoscopic examination was terminated at the level at which mucosal injury was noted; no attempt was made to pass the esophagoscope beyond the uppermost point of injury. When there was evidence of severe hypopharyngeal injury, the esophagoscope was passed only as far as the cricopharyngeal orifice. Mucosal eschar at this site was considered good evidence of esophageal burn.

Those children in whom esophagoscopy did not reveal corrosive injury were discharged within 2 or 3 days.

When a positive diagnosis of corrosive burns of the esophagus was made, corticosteroid therapy in the form of prednisolone (30 to 50 mg. per day in divided doses) was

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immediately instituted. All of these patients were treated with parenteral antibiotics; generally, penicillin was used for one week. Fluids were given on the first day only. As soon as dysphagia subsided, a soft diet was given. Corticosteroids were continued for 2 weeks at which time esophagoscopy was repeated. If the entire esophageal mucosa was completely intact at this time, the patient was discharged. When there was evidence of continued ulceration of the mucosa or when granulation tissue was noted at the site of previous injury, corticosteroid therapy was maintained. These cases were re-examined at 2-week intervals until there was complete healing of the mucosa or until early stricture formation was noted.

When the latter formation was detected, a silk string was passed, and dilatation with bougies was performed. A lumen was obtained and gradually increased in caliber until an adequate lumen was maintained with regular but infrequent dilatations.

RESULTS

In each of the 76 children presented in this study there was a history of contact with corrosive agents. In 64 there was definite evidence of corrosive burn of the lips, tongue, palate, or pharynx. In the remaining 12 there was no distinct evidence of injury to these areas, but history led us to believe that there might have been ingestion of a corrosive agent.

Of the 64 patients with oropharyngeal burns, 16 had definite evidence of esophageal burns as revealed by esophagoscopy (Table III). Of the 12 patients without evidence of oropharyngeal burns, one child (F. E.) had definite manifestation of eschar in the distal esophagus (Table II).

Of the 17 with esophageal burns all but 1 were treated with the corticosteroid, antibiotic, and soft diet regimen. Of the treated patients, one developed stricture of the hypopharynx and esophagus as well as severe scars of the lip, mouth, and tongue. Esophagoscopy on admission in this case revealed extremely severe burns. One patient (T. D.) was not treated because the examiner noted very superficial linear mucosal injury. It was felt that there was little possibility of stricture formation with this type of injury. Although this child has not had a follow-up esophagram, she is asymptomatic.

Barium esophagram was completed in 9 of the patients who were treated for corrosive injury but had no apparent stricture formation. This examination was done at varying intervals ranging from 1 to 18 months following initial treatment with corticosteroids. None of these 9 patients showed evidence of esophageal stricture or narrowing on barium examination. The other children in this series have been lost in follow-up.

DISCUSSION

Early limited esophagoscopy, within 24 hours of the accident, was performed to determine the presence or absence of esophageal injury. Some authors[1] have stated that esophagoscopy should not be performed during the first few weeks. Hanckel[2] states, "Esophagoscopy should be done on all cases, but in order to avoid perforation, not for several weeks after the burn has occurred." Davis[3] performed careful esophagoscopy and pharyngoscopy after 48 to 72 hours following initial injury. All were done under general anesthesia, and there was no morbidity or death. In our series of 17 cases, esophagoscopy in the presence of esophageal burns did not result in any complications. There were no complications in any of the cases in the series as a result of esophagos-

Table I. Agents causing corrosive injury

Agent	*Suspected burns*		*Actual burns*	
	No.	*%*	*No.*	*%*
Sodium hydroxide (Drano, lye)	65	85.5	16	94
Potassium permanganate	1	1.3	0	
Clinitest tablets	2	2.7	0	
Chlorox	1	1.3	0	
Lysol	4	5.2	1	6
Roman cleanser	1	1.3	0	
Undetermined	2	2.7	0	
Total	76	100.0	17	100.0

Table II

Patient	*Age*	*Agent*	*Burns—lip, mouth, pharynx*	*Burns—esophagus*	*Corticosteroid therapy*	*Follow-up esophagram*
1. E. E.	3	NaOH	Yes	Yes	7/16/60 (Yes)	Negative 8/12/57
2. F. E.	5	Lysol	No	Yes	12/ 7/57 (Yes)	----------
3. A. A.	2	NaOH	Yes	Yes	5/31/58 (Yes)	Severe stricture
4. K. S.	2	NaOH	Yes	Yes	11/24/59 (Yes)	----------
5. W. J.	2	NaOH	Yes	Yes	8/14/58 (Yes)	----------
6. C. C.	14	NaOH	Yes	Yes	6/24/59 (Yes)	----------
7. H. C.	3	NaOH	Yes	Yes	12/13/58 (Yes)	Negative 7/15/60
8. V. T.	1¾	NaOH	Yes	Yes	12/23/58 (Yes)	Negative 1/26/61
9. M. S.	2	NaOH	Yes	Yes	3/ 3/60 (Yes)	Negative 7/12/60
10. O. W.	1½	NaOH	Yes	Yes	9/10/59 (Yes)	Negative 7/12/60
11. B. B.	4	NaOH	Yes	Yes	9/ 5/59 (Yes)	Negative 1/12/60
12. D. T.	4	NaOH	Yes	Yes	9/18/59 (Yes)	Negative 12/ 9/59
13. E. T.	13	NaOH	Yes	Yes	2/24/60 (Yes)	Negative 4/27/60
14. L. W.	12	NaOH	Yes	Yes	8/24/59 (Yes)	Negative 7/15/60
15. L. L.	2	NaOH	Yes	Yes	1/29/58 (Yes)	----------
16. T. D.	4	NaOH	Yes	Yes (very mild)	1/29/58 (No)	----------
17. M. V.	4	NaOH	Yes	Yes	9/12/57 (Yes)	----------

copy. All children were examined under general anesthesia. In all patients in whom distinct injury was present, the esophagoscope was not passed beyond this area. Examination of the involved area was made with utmost care. We feel that if these precautions are observed, esophagoscopy within 24 hours is a safe procedure.

Certainly, these limitations make it difficult properly to assess the extent and degree of esophageal injury. It is difficult to distinguish the mild mucosal necrosis of first degree burns from the mucosal necrosis overlying second and third degree burns unless ulceration is present. Further, since we did not pass the esophagoscope beyond the uppermost site of injury, it is possible that some of these patients had more severe esophageal injury distally.

In spite of these limitations we feel there are advantages to early esophagoscopy. Through this approach it is possible to make a definite diagnosis of corrosive burns of the esophagus and thereby have a sound basis for instituting therapy. Of the 64 children with definite injury to the lips, tongue, mouth, or pharynx only 16 had esophageal injury. Thus the presence of injury in the oropharynx certainly cannot be considered a reliable criterion for the institution of therapy for esophageal burns. It is also shown that in one patient esophageal injury was present without injury in the mouth or pharynx. While this is an unusual occurrence, it emphasizes the lack of reliability of using injury of this area to diagnose esophageal injury.

A second advantage is that lengthy hospitalization or observation was eliminated in a large number (59) of children who had a history of ingestion of corrosive agents. Finally, by using corticosteroid therapy only for proved burns, we were able to evaluate better the effect of these agents in the prevention of stricture of the esophagus.

Of the 16 cases of corrosive injury of the esophagus treated with corticosteroids, only one developed stricture. Corticosteroid therapy for the prevention of fibrosis has had general acceptance in the last decade. In 1950, Spain, Molomut, and Haber[4] demonstrated that cortisone inhibited granulation tissue formation in mice. However, significant inhibition of granulation tissue formation occurred only when the cortisone was

Table III

	No.	*%*
Suspected burns (total cases)	76	100
Burns—mouth	64	84
Actual burns—esophagus	17	22
Strictures	1	1.3

given during the early stage of initiation of the repair stimulus. Rosenburg and colleagues[5] demonstrated that cortisone, by inhibiting fibroplasia, greatly diminished the tendency to scar tissue contractures after localized sodium hydroxide burn in the rabbit esophagus. In 1952, Weiskoff[6] induced lye burns of the esophagus in dogs and demonstrated the effectiveness of early corticosteroid and antibiotic therapy in preventing stricture. Recent clinical trials have given encouraging results.[4, 7, 8]

Our results seem to be very heartening. However, certain facts should be considered. As noted earlier, it is possible to make a definite diagnosis of esophageal burns with early esophagoscopy. However, we are not always certain of the degree of involvement of the esophageal wall. Thus, it is possible that the esophageal lesions which, on visual inspection appeared to be second or third degree burns, may have been limited to the mucosa. Such mucosal lesions generally do not cause stricture formation. Therefore, some of the esophageal lesions noted in our series may have healed spontaneously. Finally, in one case in this series (A. A.) where severe esophageal injury was present, considerable stricture formation occurred despite the use of corticosteroids. This has also been observed in 2 adults who were treated on our service after ingestion of large amounts of lye in suicide attempts. It is apparent that the true value of corticosteroids will be determined only when a more definite method of grading esophageal lesions is available.

It is of interest to compare the incidence of esophageal stricture in our series with that recorded by Fati, Marchand, and Croushore.[9] In their experience, "Approximately 50 per cent of all patients admitted with acute caustic soda poisoning develop esophageal strictures." Ray and Morgan,[7] reporting on their experience in use of corticosteroid therapy in corrosive esophageal injury, stated that their experiences prior to the use of corticosteroids "led them to expect 25 per cent to 50 per cent" of the patients to develop esophageal strictures. In our 17 cases of proved burns only one (5.2 per cent) developed a stricture. Of all the 76 children who are represented, the incidence of stricture is only 1.3 per cent. We feel that the difference is the result of greater accuracy in diagnosis afforded by early esophagoscopy and by prompt institution of corticosteroid therapy.

CONCLUSIONS AND SUMMARY

From a study of 76 children with a history of contact with a corrosive agent, we arrived at the following conclusions:

1. Corrosive burns of the mouth and pharynx do not necessarily indicate the presence of esophageal burns.

2. Limited esophagoscopy within 24 hours after ingestion of corrosive agents is a safe procedure.

3. Early esophagoscopy provides a positive diagnosis and a sound basis for institution of corticosteroid therapy in corrosive burns of the esophagus.

4. In children with presumptive evidence of esophageal burns, early esophagoscopy may reveal absence of injury and eliminate lengthy hospitalization and treatment.

5. The use of corticosteroids within 48 hours of injury is probably valuable in the prevention of stricture following corrosive burns of the esophagus.

REFERENCES

1. Holinger, Paul H., and Johnston, K. C.: Caustic Stricture of the Esophagus, Illinois M. J. **98:** 246, 1950.
2. Hanckel, R. W., Jr.: Some Observations Concerning the Salzer Method of Treatment of Lye Burns of the Esophagus, South. M. J. **39:** 263, 1956.
3. Davis, M. V.: Early and Late Management of Caustic Burns of the Esophagus, Am. Surgeon **22:** 308, 1956.
4. Spain, D., Molomut, N., and Haber, A.: Biological Studies on Cortisone in Mice, Science **112:** 335, 1950.
5. Rosenburg, N., Kunderman, P. J., Vroman, L., and Moolten, S. E.: Prevention of Experimental Lye Strictures of the Esophagus by Cortisone, A. M. A. Arch. Surg. **63:** 147, 1951.
6. Weiskoff, A.: Effects of Cortisone on Experimental Lye Burn, Ann. Otol., Rhin. & Laryng. **61:** 681, 1952.

7. Ray, E. S., and Morgan, D. L.: Cortisone Therapy of Lye Burns of the Esophagus, J. PEDIAT. **49:** 394, 1959.
8. Yurich, E. L.: Early Diagnosis and Treatment of Corrosive Burns of the Esophagus, Laryngoscope **69:** 131, 1959.
9. Fatti, L., Marchand, P., and Croushore, G. R.: The Treatment of Caustic Strictures of the Esophagus, Surg. Gynec. & Obst. **102:** 195, 1957.
10. Alford, B. R., and Harris, H. H.: Chemical Burns of the Mouth, Pharynx and Esophagus, Ann. Otol., Rhin. & Laryng. **68:** 122, 1959.
11. Daly, F., and Cardona, J. C.: Corrosive Esophagitis, Am. J. Surg. **93:** 242, 1957.
12. Rosenburg, N., Kunderman, P. J., Vroman, L., and Moolten, S. E.: Prevention of Experimental Esophageal Stricture by Cortisone II. Control of Suppurative Complications by Penicillin, A. M. A. Arch. Surg. **66:** 593, 1953.
13. Spain, D., Molomut, N., and Haber, A.: The Effect of Cortisone on the Formation of Granulation Tissue in Mice, Am. J. Path. **26:** 710, 1950.
14. Waggoner, Lyle G.: Diagnosis and Management of Chemical Burns of the Esophagus, Laryngoscope **68:** 1790, 1958.

Blood plasma levels and urinary excretion of ascorbic acid before and after a test dose in children with severe thalassemia

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ALTHOUGH the role of ascorbic acid in blood formation has not been fully established, evidence has been presented that this vitamin is indirectly concerned with hemopoiesis. The synthesis of folic acid in vivo is stimulated by folic acid and by ascorbic acid,[1] and Nichol and Welch[2] have shown that the addition of ascorbic acid to liver and kidney systems in vitro augments the conversion of folic acid to folinic acid. These authors also found that the urinary excretion of folinic acid in man and in the rat was increased by administration of folic acid and increased still further by the addition of ascorbic acid. The fact that anemia may occur in scurvy does not prove that ascorbic acid is important for blood formation, since anemia may result from hemorrhage or deficiency of blood-forming substances other than ascorbic acid. Crandon,[3] who remained for 6 months on a diet totally devoid of ascorbic acid but supplemented with other vitamins, developed manifest scurvy but no anemia. Nevertheless, megaloblastic anemia has been produced in addition to scurvy in monkeys fed milk diets without added ascorbic acid.[4-6] This anemia was very similar to the megaloblastic anemia of infancy,[7-9] and it should be pointed out that many infants with this type of anemia had also been fed milk not supplemented with ascorbic acid.[10, 11] In the scorbutic, megaloblastic monkey the bone marrow was restored to normal with either folic acid or L-ascorbic acid, but it was not affected by D-ascorbic acid.[6] Folic acid and L-ascorbic acid also caused accumulation of folic acid and folinic acid in the liver.[6]

During the last few years cases have been reported of megaloblastic anemia complicating sickle cell anemia, sickle cell–hemoglobin C anemia,[12] and thalassemia[13-16] which responded to liver extract[13] or folic acid,[12, 14, 16] indicating a deficiency of these factors in such patients.

Although instances of thalassemia with

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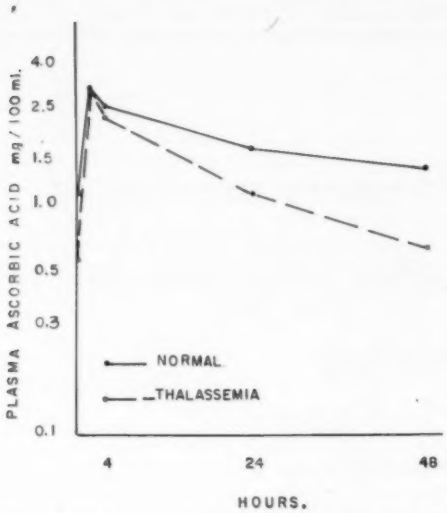

Fig. 1. The mean plasma ascorbic acid levels before and after an intramuscular injection of 50 mg. per kilogram in normal children and in children with severe thalassemia.

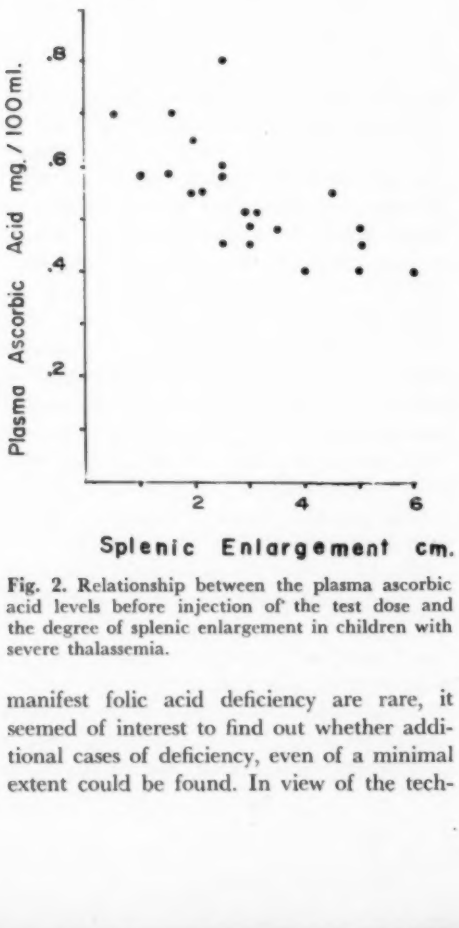

Fig. 2. Relationship between the plasma ascorbic acid levels before injection of the test dose and the degree of splenic enlargement in children with severe thalassemia.

manifest folic acid deficiency are rare, it seemed of interest to find out whether additional cases of deficiency, even of a minimal extent could be found. In view of the technical difficulties involved in the estimation of folic acid and of the interrelationship between folic acid and ascorbic acid, it seemed possible that some indirect information could be obtained by studying the response of patients with thalassemia to an intramuscular administration of ascorbic acid.

This paper records the results of studies in normal children and in children with severe thalassemia.

MATERIAL AND METHODS

Plasma ascorbic acid levels were determined in 22 children with severe thalassemia and in 12 normal children before, 2, 4, 24, and 48 hours following an intramuscular injection of 50 mg. per kilogram of body weight of ascorbic acid. In 5 of the anemic and 5 of the normal children the urinary excretion of ascorbic acid was measured before and after the injection of the test dose.

The diagnosis of thalassemia was based on: (1) the clinical observations which included pallor, splenomegaly, underdevelopment, and, in some children, a mongoloid facies; (2) the hematologic changes of severe anemia, normoblastosis, anisocytosis, poikilocytosis, hypochromia and a high proportion of alkali-resistant hemoglobin; and (3) the trait of thalassemia in the parents.

The control group consisted of children of the same age group as the children with severe thalassemia (5 months to 13 years). They were well developed and, at the time of examination, had no fever or other evidence of infection, splenic enlargement, or anemia.

Since the blood and urine levels of ascorbic acid may be affected by the preceding dietary intake,[17] both the normal and the anemic groups of children were investigated 3 days after admission to the hospital during which time they were fed a diet totally devoid of vitamin C. It has been shown that, following a test dose, it takes about 2 days for the plasma levels of ascorbic acid to return to normal.[18] A 3-day period was, therefore, thought to be long enough for the plasma level to return to normal in any instance in which it might have been raised

Table I. Plasma ascorbic acid levels before and after injection of 50 mg. per kilogram in 12 normal children (mg./100 ml.)

Case No.	Age	Hours				
		0	2	4	24	48
1	5 months	1.20	2.80	2.40	1.60	1.35
2	8 months	0.93	3.00	2.60	2.30	1.60
3	9 months	1.08	3.20	2.35	1.20	1.20
4	12 months	1.00	2.90	2.50	1.70	1.30
5	15 months	1.05	2.85	2.40	1.80	1.20
6	20 months	1.15	3.30	2.80	1.45	1.40
7	3 years	1.00	3.00	2.80	1.50	1.00
8	5 years	1.13	3.00	2.50	1.70	1.50
9	6 years	0.85	2.80	2.10	1.50	1.30
10	9 years	1.35	3.20	2.80	1.60	1.30
11	9 years	1.20	3.10	2.40	1.90	1.40
12	13 years	1.25	3.10	2.90	1.60	1.55
	Means	1.10	3.02	2.54	1.65	1.34
	S.D.	±0.10	±0.17	±0.23	±0.26	±0.17

because of a previously high intake. During the test, the children were fed the same diet and were allowed water ad libitum.

Urine was collected by an indwelling catheter before and at frequent intervals during the 48 hours following the intramuscular injection of ascorbic acid. At the end of each period of collection, which ranged from 2 to 6 hours, air was introduced into the bladder by a syringe and the bladder was completely emptied by manual suprapubic pressure. The exact time of the beginning and the end of each period of collection was carefully noted.

Plasma ascorbic acid was determined according to the volumetric method of Farmer and Abt.[19] Five milliliters of fresh heparinized blood, drawn from the antecubital or jugular vein in the usual way, was centrifuged and the supernatant plasma separated as quickly as possible. The plasma proteins were precipitated with 5 ml. of a 5 per cent solution of metaphosphoric acid added to 2 ml. of plasma which had been diluted with 4 ml. of distilled water. After centrifugation, ascorbic acid in the clear supernatant fluid was titrated with 2,6-dichlorophenol-indophenol sodium.* Two milliliters of supernatant was placed in a test tube. A blank was prepared by placing 1 ml. of the 5 per cent metaphosphoric acid and 1 ml. of distilled water in another test tube. The 2,6-dichlorophenol-indophenol sodium solution was then added from a 5 ml. microburet graduated to 0.02 ml. to each tube, dropwise, until a pink color was obtained that persisted for 30 to 60 seconds.

The 2,6-dichlorophenol-indophenol sodium solution was prepared by dissolving 1 tablet (1.6 mg.) in 100 ml. of freshly distilled water and was standardized with a known solution of ascorbic acid, so that 1 ml. of the solution was reduced by 0.01 mg. of ascorbic acid. Both the standard solution of ascorbic acid and the 2,6-dichlorophenol-indophenol sodium solution were prepared immediately before use. The blood plasma ascorbic acid was calculated from the following formula:

$$\frac{\text{difference between titrations} \times 0.01 \times 100}{0.4} = \text{mg./100 ml.}$$

The concentration of ascorbic acid in the urine was determined by titrating an acidified sample of urine with 2,6-dichlorophenol-indophenol sodium according to the method of Harris and Ray.[19] Ten milliliters of 2,6-dichlorophenol-indophenol sodium solution, prepared as for the determination of ascorbic acid in the plasma, was pipetted into a porcelain dish. Five per cent acetic acid was added drop-wise until a red color appeared. A 5 ml. microburet graduated to 0.02 ml. was filled with urine, which was added to the dye solution in the dish until the red color disappeared. The urine ascorbic acid was calculated as follows:

$$\frac{10}{\text{ml. of urine titrated}} = \text{mg./100 ml.}$$

The titration of ascorbic acid in both the plasma and the urine was completed within 2 minutes.

RESULTS

Plasma ascorbic acid levels.

A. Normal children. The plasma ascorbic acid levels in 12 normal children before and after the intramuscular injection of ascorbic

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Table II. Plasma ascorbic acid levels before and after an intramuscular injection of 50 mg. per kilogram in 22 children with severe thalassemia (mg./100 ml.)

Case No.	*Age*	*Hemoglobin (Gm./100 ml.)*	*Alkali resistant hemoglobin (%)*	*Splenic enlargement (cm.)*	*Hours*				
					0	*2*	*4*	*24*	*48*
1	2 months	6.60	65	1.5	0.58	2.90	2.20	1.10	0.65
2	4 months	6.39	46	3.0	0.50	2.80	2.10	1.05	0.55
3	6 months	5.40	60	2.0	0.65	3.00	2.20	1.00	0.70
4	7 months	6.13	68	3.0	0.50	2.85	2.40	1.05	0.65
5	8 months	6.13	45	3.0	0.50	3.00	2.80	1.10	0.55
6	9 months	5.15	36	2.5	0.80	3.20	2.70	1.20	0.90
7	12 months	6.13	41	0.5	0.70	2.90	1.90	1.05	0.70
8	13 months	5.15	60	2.0	0.55	3.10	2.45	1.10	0.65
9	13 months	4.90	67	2.5	0.60	3.00	2.10	1.50	0.70
10	14 months	5.15	70	2.0	0.55	3.00	2.60	1.10	0.65
11	14 months	4.00	85	2.5	0.58	2.80	2.00	1.20	0.60
12	16 months	2.80	75	5.0	0.40	2.90	2.20	1.00	0.45
13	19 months	3.80	43	4.0	0.40	2.80	2.00	0.60	0.45
14	23 months	4.20	48	3.0	0.45	2.90	2.50	1.15	0.50
15	23 months	5.30	28	1.0	0.58	3.20	1.80	1.00	0.65
16	2 years	5.40	34	1.5	0.70	2.90	2.60	1.20	0.80
17	2 years	5.15	86	3.5	0.48	3.05	2.75	1.15	0.70
18	3 years	6.13	68	5.0	0.45	2.90	2.30	1.10	0.50
19	4½ years	4.00	32	2.5	0.45	3.00	2.00	0.95	0.60
20	6 years	6.39	48	5.0	0.48	3.20	2.80	0.90	0.55
21	8 years	6.13	26	4.5	0.55	2.85	2.00	0.90	0.50
22	12 years	4.00	64	6.0	0.40	2.80	2.00	1.20	0.60
				Means	0.54	2.95	2.29	1.07	0.62
				S.D.	±0.10	±0.24	±0.31	±0.17	±0.10

acid are illustrated in Table I. Before the injection the levels ranged from 0.85 to 1.35 mg. per 100 ml. of plasma, being closely comparable to those reported by other investigators for normal adults[17, 20, 21] or normal children.[22]

There was a threefold rise in the plasma levels 2 hours following the injection, after which the concentrations gradually decreased. The ratio between the levels at 0, 2, 4, 24, and 48 hours was about 1:3:2.5:1.6:1.2, the plasma clearance being almost linear between 4 and 48 hours (Fig. 1). At the end of 48 hours the plasma ascorbic acid had fallen to a concentration which was, on the average, about 20 per cent higher than that observed before the injection.

B. Children with thalassemia. Table II shows the plasma ascorbic acid concentrations in 22 children with severe thalassemia before and after the intramuscular injection of the test dose. It can be seen that the concentrations before the injection were invariably lower than those of the normal children, ranging from 0.4 to 0.8 mg. per 100 ml. There was no relationship between the plasma ascorbic acid levels and the severity of the anemia as judged by the concentration of hemoglobin in whole blood ($r = 0.380 < 0.423$, $n\text{-}2 = 20$, $P = 0.05$) but the plasma levels tended to be somewhat lower in the children with considerable splenomegaly than in those in whom the spleen was moderately or slightly enlarged ($r = -0.657 > -0.537$, $n\text{–}2 = 20$, $P = 0.01$) (Fig. 2).

The data in Table II also show that 2 hours after the intramuscular dose the plasma ascorbic acid, though starting at a lower level, rose to concentrations which were almost identical with those of the normal children. The ratio between the levels at 0, 2, 4, 24, and 48 hours was about 1:6:4:2:1.1. Just as in the normal children, the plasma clearance was linear between 4 and 48 hours (Fig. 1), but the mean concentration of ascorbic acid at 4,* 24, and 48 hours was

*$t = 2.466 > 2.042$, $n\text{–}2 = 32$, $P = 0.05$.

significantly lower in the anemic children (Tables I and II, Fig. 1), showing that the rate of removal of ascorbic acid from the plasma in the children with thalassemia was greater than in the normal ones. At the end of 48 hours, the plasma level of ascorbic acid in these children was, on the average, very little higher than that obtained before the injection of the test dose.

These results show that an equivalent amount of ascorbic acid produced an initial rise in the plasma levels which was more pronounced in thalassemia and that this was followed by a more rapid removal of the vitamin from the blood.

Urinary excretion of ascorbic acid.

A. Normal children. The urinary excretion of ascorbic acid before and after the injection of the test dose in 5 of the normal children is given in Table III. The test dose caused a considerable increase in the elimination of ascorbic acid; the rate of urinary excretion reached its peak at 2 hours after which it declined, rather abruptly (Fig. 3), but had not returned to its base line 48 hours after the injection.

Fig. 4 illustrates the urinary excretion of ascorbic acid, expressed as a percentage of the test dose, between 0 and 48 hours. Two hours after the injection, the normal children had eliminated about one third of the dose of ascorbic acid and this is to be corrected with the rapid increase in the excretion of ascorbic acid shown in Table III. After this time, they continued to excrete large quantities of the vitamin, though at a gradually declining rate till, at 48 hours, 70 per cent of the ascorbic acid administered had been excreted. The percentage of the injected dose retained presumably reflects the amount of ascorbic acid normally destroyed in the body through metabolic changes.[17, 20]

B. Thalassemia. Table IV illustrates the urinary excretion of ascorbic acid before and after the injection of the test dose in 5 of the children with severe thalassemia. The rate of excretion was significantly lower than normal before the injection, but the test dose produced an increase in the elimination of ascorbic acid which, at 2 hours, was of the same order as that observed in the normal children. After this time, however, the rate

Table III. The urinary excretion, U.V. (mg./min. 1.73 m²), of ascorbic acid before and after an intramuscular injection of 50 mg. per kilogram in 5 normal children

Case No.	*Time (hours)*												
	0	*2*	*4*	*10*	*14*	*17*	*20*	*24*	*28*	*34*	*38*	*44*	*48*
4	0.030	4.23	1.89	0.55	0.352	0.189	0.134	0.085	0.071	0.076	0.071	0.060	0.055
5	0.019	4.14	2.39	0.63	0.276	0.152	0.127	0.110	0.110	0.097	0.069	0.073	0.064
8	0.017	3.94	1.90	0.93	0.528	0.310	0.176	0.157	0.108	0.077	0.075	0.042	0.040
10	0.021	5.19	2.61	1.01	0.427	0.176	0.163	0.140	0.138	0.117	0.116	0.092	0.082
12	0.022	4.59	2.01	1.00	0.536	0.402	0.223	0.164	0.134	0.113	0.116	0.094	0.089
Means	0.022	4.42	2.16	0.82	0.420	0.240	0.160	0.130	0.110	0.100	0.090	0.070	0.066

Table IV. The urinary excretion, U.V. (mg./min. 1.73 m²), of ascorbic acid before and after an intramuscular injection of 50 mg. per kilogram in 5 children with severe thalassemia

Case No.	*Time (hours)*												
	0	*2*	*4*	*10*	*14*	*17*	*20*	*24*	*28*	*34*	*38*	*44*	*48*
10	0.0050	4.47	1.33	0.29	0.09	0.077	0.049	0.018	0.015	0.013	0.011	0.010	0.008
13	0.0046	3.68	1.15	0.43	0.08	0.035	0.027	0.018	0.013	0.040	0.010	0.009	0.005
18	0.0059	3.91	1.21	0.22	0.11	0.054	0.051	0.040	0.038	0.025	0.024	0.013	0.007
21	0.0048	4.81	1.94	0.23	0.04	0.034	0.025	0.016	0.014	0.016	0.016	0.013	0.012
22	0.0090	4.17	1.56	0.35	0.19	0.068	0.040	0.035	0.035	0.023	0.023	0.016	0.013
Means	0.0060	4.21	1.44	0.30	0.10	0.050	0.040	0.025	0.023	0.018	0.016	0.012	0.009

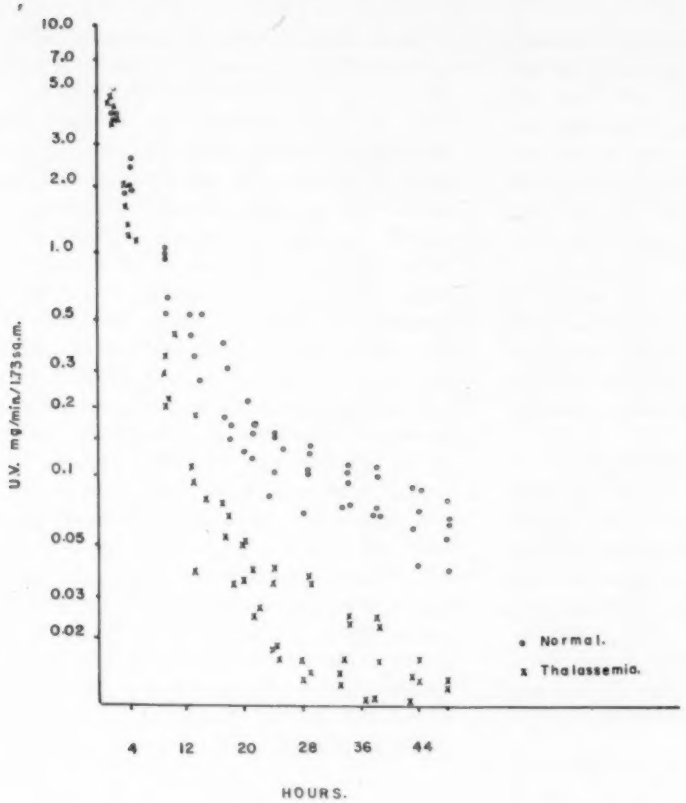

Fig. 3. The urinary excretion of ascorbic acid after an intramuscular injection of 50 mg. per kilogram in normal children and children with severe thalassemia.

Fig. 4. The percentage of the test dose excreted in the urine after the intramuscular injection of 50 mg. of ascorbic acid per kilogram of body weight.

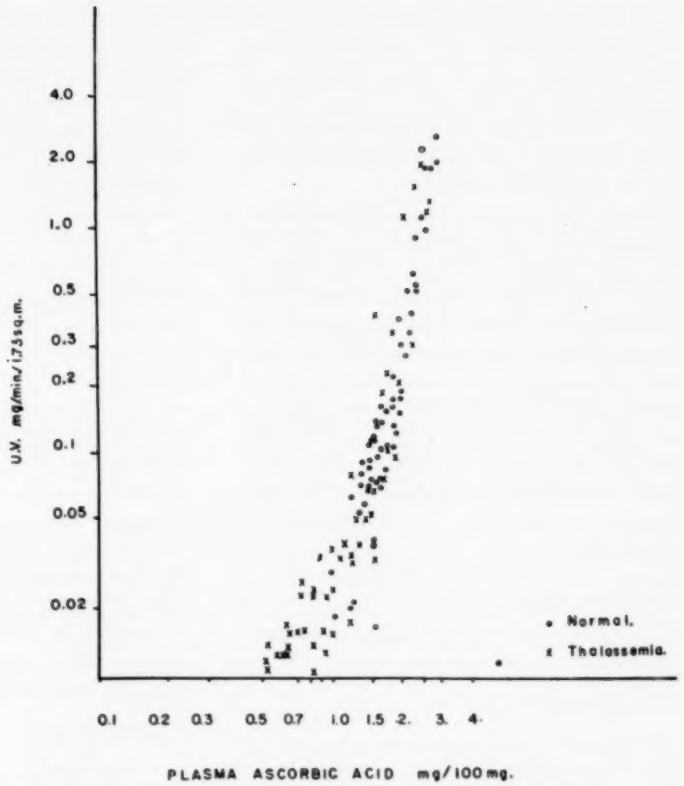

Fig. 5. Variations in the rate of urinary excretion with the plasma concentrations of ascorbic acid. Both scales are logarithmic.

of excretion declined more rapidly in the anemic children (Fig. 3) and at the end of 48 hours had returned to a value which was closer to its base line than it was in the normal children (Tables III and IV).

Fig. 5 shows the rates of urinary excretion of ascorbic acid in the normal and the anemic children plotted against the plasma concentration, both on logarithmic scales. It can be seen that with plasma concentrations between 1.25 and 3 mg. per 100 ml., at any given plasma concentration, the anemic children excreted the same amount of ascorbic acid as did the normal children. Since the rate of urinary excretion of any substance divided by its plasma concentration gives the renal clearance of the substance, these results also show that, when additional ascorbic acid was injected in amounts which were sufficient to raise the plasma levels to more than 1.25 mg. per 100 ml., at equivalent concentrations of the plasma, the latter was cleared by the kidney at almost exactly the same rate in both the normal and the anemic children. In other words, had the plasma levels of ascorbic acid been the same in the normal and the anemic children throughout the 48-hour period of observation, their rate of urinary excretion of the vitamin would have been almost identical. Nevertheless, except for the first 2 hours, the plasma levels were lower in the children with thalassemia. That this had not resulted from a greater increase in the renal elimination of ascorbic acid is demonstrated by the fact that the rates of excretion during the first few hours were about the same in both the normal and the anemic children (Fig. 3). On the con-

trary, it appears that it was the greater fall in the plasma levels which produced a more striking decrease in the urinary excretion of ascorbic acid in the children with thalassemia. These findings indicate a normal renal response to an increased load of ascorbic acid in the anemic children.

Fig. 4 shows that 4 hours after the injection both the normal and the anemic children had eliminated about 40 per cent of the test dose. After this time, however, the percentage of ascorbic acid excreted by the anemic children was considerably less than that excreted by the normal children till, at the end of 48 hours, the former had excreted only about half of the injected dose. The over-all retention of ascorbic acid was thus shown to be appreciably greater in the children with thalassemia. This observation, together with that of a more rapid fall in the plasma levels, shows that the tissues were removing the vitamin from the blood stream more actively in the anemic than in the normal children.

DISCUSSION

The results of this study show that children with severe thalassemia differed from normal children (1) in their plasma levels and their urinary excretion of ascorbic acid and (2) in their response to an intramuscular injection of a test dose of the vitamin.

The plasma concentrations and the urinary excretion of ascorbic acid in the children with thalassemia were invariably lower than those of normal children. Although it has been pointed out that the plasma levels do not always reflect the vitamin C status of an individual,[17] the range of values obtained from the anemic children together with their low urinary excretion suggests a mild deficiency.[17, 20, 23] This is corroborated by the observation that, during a saturation test, the rate of removal of the injected ascorbic acid from the plasma was more rapid in the thalassemic than in the normal children and that the over-all retention of the vitamin was appreciably greater in the anemic children.

It is probable that the children with thalassemia retained a greater proportion of the injected dose in order to meet an increased rate of hemopoiesis. Nevertheless, saturation tests with ascorbic acid reflect the degree of tissue saturation of the body as a whole. It is, therefore, equally probable that it was all the tissues, not only those concerned with blood formation, which were deficient in the anemic children.

Since both the normal and the children with anemia of the present study were drawn from the same population with a presumably similar intake of ascorbic acid, it is rather unlikely that the deficiency of the anemic children was due to a reduced dietary intake of the vitamin. Unless these children are unable to absorb ascorbic acid from the gastrointestinal tract, this deficiency must be attributed to increased use of the vitamin. Whether more ascorbic acid is used only by the overactive blood-forming tissues, or by all the body tissues, is a matter which cannot be settled from the results of this study. Investigation of the vitamin C status in other anemias with increased hemopoiesis will probably help to answer this question.

SUMMARY

1. The plasma ascorbic acid levels were determined in 22 children with severe thalassemia and in 12 normal children before and after an intramuscular injection of 50 mg. per kilogram of ascorbic acid. In 5 of the normal and 5 of the anemic children the urinary excretion of ascorbic acid before and after the test dose was also investigated.

2. Before the injection of the test dose, the plasma ascorbic acid levels were invariably lower in the children with thalassemia than in the normal ones, and especially so in those with considerable splenomegaly. The test dose produced a greater initial rise, followed by a more rapid fall in the plasma levels of the anemic subjects.

3. The urinary excretion of ascorbic acid before the test dose was considerably lower than normal in the children with thalassemia. The test dose produced a very similar initial rise in the renal elimination of the vitamin in both the normal and the anemic children, but this was followed by a decline in the rate

of excretion which was more rapid in the anemic children. The over-all retention of injected ascorbic acid was considerably greater in the children with thalassemia.

4. These observations suggest a mild ascorbic acid deficiency in children with severe thalassemia. The significance of this deficiency is discussed.

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REFERENCES

1. Dietrich, L. S., Nichol, C. A., Monston, W. J., and Elvehjem, C. A.: Observations on the Interrelation of Vitamin B_{12}, Folic Acid and Vitamin C in the Chick, J. Biol. Chem. **181:** 915, 1949.
2. Nichol, C. A., and Welch, A. D.: Synthesis of Citrovorum Factor From Folic Acid by Liver Slices; Augmentation by Ascorbic Acid, Proc. Soc. Exper. Biol. & Med. **74:** 52, 1950.
3. Crandon, J. H., Lund, C. C., and Dill, D. B.: Experimental Human Scurvy, New England J. Med. **223:** 353, 1940.
4. May, C. D., Sundberg, R. D., Schaar, F., Lowe, C. U., and Salmon, R. J.: Experimental Nutritional Megaloblastic Anemia: Relation of Ascorbic Acid and Pteroylglutamic Acid, A.M.A. Am. J. Dis. Child. **82:** 282, 1951.
5. Proehl, E. C., and May, C. D.: Experimental Nutritional Megaloblastic Anemia and Scurvy in the Monkey. III. Protoporphyrin, Coproporphyrin, Urobilinogen and Iron in Blood and Excreta, Blood **7:** 671, 1952.
6. May, C. D., Hamilton, A., and Stewart, C. T.: Experimental Megaloblastic Anemia and Scurvy in the Monkey. IV. Vitamin B_{12} and Folic Acid Compounds in the Diet, Liver, Urine and Feces and Effects of Therapy, Blood **7:** 978, 1952.
7. Veeneklaas, G. M. H.: Ueber megalozytäre Mangelanämien bei Kleinkindern, Folia haemat. **65:** 303, 1941.
8. Zuelzer, W. W., and Ogden, F. N.: Megaloblastic Anemia in Infancy: A Common Syndrome Responding Specifically to Folic Acid Therapy, Am. J. Dis. Child. **71:** 211, 1946.
9. Amato, M.: Clinical Histories and Hematologic, Biochemical and Pathogenic Studies on 25 Cases of Hyperchromatic Megaloblastic Anemia Observed in Young Infants, Pediatria **54:** 71, 1946.
10. Luhby, A. L., and Wheeler, W. E.: Megaloblastic Anemia of Infancy: II. Failure of Response to Vitamin B_{12} and the Metabolic Rôle of Folic Acid and Vitamin C, Health Center J., Ohio State University **3:** 1, 1949.
11. May, C. D., Nelson, E. N., Lowe, C. U., and Salmon, R. J.: Pathogenesis of Megaloblastic Anemia in Infancy. An Interrelationship Between Pteroylglutamic Acid and Ascorbic Acid, Am. J. Dis. Child. **80:** 191, 1950.
12. Jonsson, U., Roath, O. S., and Kirkpatrick, C. I. F.: Nutritional Megaloblastic Anemia Associated With Sickle-Cell States, Blood **14:** 535, 1959.
13. Crosby, W. H., and Sacks, H. J.: The Coincidence of Mediterranean Anemia and Pernicious Anemia in a Young Sicilian, Blood **4:** 1267, 1949.
14. Goldberg, M. A., and Schwartz, S. O.: Mediterranean Anemia in a Negro Complicated by Pernicious Anemia of Pregnancy, Blood **9:** 648, 1954.
15. Chanarin, I., Dacie, J. V., and Mollin, D. L.: Folic Acid Deficiency in Hemolytic Anemia, Brit. J. Haemat. **5:** 245, 1959.
16. Fessas, P.: Thalassemia and the Alterations of the Hemoglobin Pattern. Abnormal Hemoglobins. A Symposium, Oxford, 1959, Blackwell Scientific Publications, p. 134.
17. West, E. S., and Todd, W. R.: Textbook of Biochemistry, New York, 1955, The Macmillan Company.
18. Markidou, A.: Blood Plasma Levels of Ascorbic Acid in Infantile Acute Dehydration and Infantile Malnutrition Before and After a Test Dose, M.D. Thesis, Athens, 1953.
19. Kolmer, J. A., Spaulding, E. H., and Robinson, H. W.: Approved Laboratory Technic, New York, 1951, Appleton-Century-Crofts, Inc.
20. Goodman, L. S., and Gilman, A.: The Pharmacological Basis of Therapeutics, ed. 2, New York, 1956, The Macmillan Company.
21. Freeman, J. T., Hafkesbring, R., and Caldwell, E. K.: Comparative Study of Ascorbic Acid Levels in Gastric Secretion, Blood, Urine and Saliva, Gastroenterology **18:** 224, 1951.
22. Boi, G.: Ascorbic Acid Metabolism in Healthy Children With Particular Consideration of the Oxidation-Reduction Phenomenon in Relation to Age and Nourishment, Ann. Ital. Pediat. **7:** 349, 1954.
23. Levinson, S. A., and MacFate, R. P.: Clinical Laboratory Diagnosis, Philadelphia, 1951, Lea & Febiger.

Anomalous inferior vena cava with azygos continuation (infrahepatic interruption of the inferior vena cava)

Report of 15 new cases

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IN A PREVIOUS report[1] we described a single case of anomalous inferior vena cava with azygos continuation ("absent inferior vena cava") and reviewed 7 other recent cases from the literature. In the intervening time we have encountered 15 additional cases. When coupled with additional published reports, these cases permit a more adequate evaluation of this venous anomaly, particularly with regard to its incidence, its anatomic variants, and its association with other vascular and cardiac anomalies.

CASE MATERIAL

The 15 cases of absent inferior vena cava were encountered among approximately 2,500 new patients with congenital heart defects seen over a 6-year period at the University of Minnesota Hospitals. The pertinent clinical materials are shown in Table I. In 3 cases, complications of pregnancy had occurred—minimal bleeding in the first trimester in 2, hypertension in the first two trimesters in 1. In one of the former the mother had been on thyroid therapy for mild hypothyroidism. There were 28 siblings born earlier (one was stillborn, one had a clubfoot). Three siblings born later and 6 step-siblings were all normal. The parents were free of cardiac defects. One child had a normal twin, presumably identical. Three normal children had been born to the one adult patient. Birth dates showed no unusual seasonal distribution.

Cases from the recent medical literature are listed in Table II, and include only those with accompanying clinical information.

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CLINICAL FEATURES

In the present series the incidence of this anomaly in patients with congenital heart defects is 0.6 per cent. Kjellberg and coworkers[17] noted the anomaly in 3 of 668 such patients, and Loogen and Rippert[15] encountered 2 cases in 1,000 patients having cardiac catheterization. This is considerably less than the minimal 3.0 per cent estimate of the incidence of persistent left superior vena cava in patients with congenital heart defects.[12] Our clinical experience suggests the incidence of the latter to be in the range of 5 to 10 per cent. Similarly, the incidence of anomalous inferior vena cava with azygos continuation in individuals without cardiac defects is apparently considerably less than the corresponding figure of 0.3 per cent for persistent left superior vena cava.[19, 20, 21] Thus, Adachi[22] found no cases in 1,055 autopsies.

Patients with this form of anomalous inferior vena cava have generally had cardiac defects that produce cyanosis. The commonly encountered defects in this series (Table I) and in cases reported by others (Table II) include cor biloculare, atrioventricular canal, anomalously connecting pulmonary veins, double outflow right ventricle, large atrial septal defect, pulmonary stenosis or atresia, or combinations of these. Abnormal positions of the heart (mirror-image dextrocardia, dextroversion, situs inversus with levocardia or levoversion) and partial inversion of the abdominal viscera (heterotaxia) are common, as well as anomalies of the superior vena cava (bilateral superior venae cavae, in particular). However, anomalous inferior vena cava with azygos continuation may occur as an isolated and benign anomaly, and under such circumstances is not likely to be detected. Cases have been described as incidental autopsy findings in patients up to 91 years of age.[23]

Auscultatory and electrocardiographic findings in patients with this anomaly have been related to the associated cardiac defects rather than to the venous anomaly itself. Roentgenograms likewise have been of little diagnostic aid, except that an enlarged azygos vein may produce a protuberance at its junction with the superior vena cava.[10, 24] This sign was helpful in initially suspecting the diagnosis only in Case 8, and this was an older child. In cases with hemiazygos continuation into a left superior vena cava the latter vessel may produce a widened mediastinal shadow on the left. Throughout this paper, the term "hemiazygos" will be used to indicate that member of the paired azygos system that connects with the cardinal system contributing to the formation of the oblique vein of the arterial atrium, irrespective of the position of the heart.

Whereas autopsy examination was the means of diagnosis of earlier-described cases (such as the 20 cases compiled from the literature in 1901 by Dwight[23]), recently reported cases have generally been recognized as a result of special diagnostic studies, particularly angiocardiography (Tables I and II). This tends to bias the case material, inasmuch as angiocardiography is performed primarily in patients with cyanotic forms of congenital heart defects, and by way of the leg veins in infants and young children with marked cardiac disability. Thus, Stackelberg and others[9] found 2 cases among 100 patients having angiocardiography performed from the malleolar vein. An increasing number of recent cases have been initially detected by anomalous passage of the catheter at right heart catheterization.

The angiocardiographic patterns of azygos and hemiazygos continuation are shown in Figs. 1 to 4, with the typical "candy cane" appearance best seen in the lateral projection (Fig. 2).

The possibility of this anomaly must always be borne in mind at the time of right heart catheterization, particularly if the procedure is performed by way of the saphenous vein. The operator may believe he is advancing the catheter tip from the inferior vena cava, "through the right atrium," and into the superior vena cava. The fallacy of this interpretation is easily recognized by the limited lateral movements of the catheter tip in the "right atrium" (azygos vein), and by the persistent posterior location of the catheter

Table I. Case material

Case No.	*Sex*	*Status*	*Autopsy*	*Special studies*		*Position of heart*	*Position of abdominal viscera*
				Angiocardiography	*Heart catheterization*		
1§	M	Died 4 months	Yes	Yes‡	No	Dextroversion	Normal
2	M	Living 5 years	—	Yes‡	Yes	Normal	Normal
3	F	Living 5 years	—	Yes‡	Yes	Left-sided	Inverted
4	M	Died 3 months	Yes	Yes‡	Yes	Normal	Normal
5	F	Died 2 years	Yes	Yes‡	Yes	Normal	Normal
6	F	Living 1 month	—	Yes‡	Yes	Left-sided	Stomach on right
7	M	Died	Yes	Yes‡	No	Mirror-image dextrocardia	Incomplete inversion
8	M	Living 11 years; operated upon	—	Yes	Yes‡	Normal	Normal
9	F	Living 3 months	—	Yes	Yes‡	Normal	Normal
10	M	Living 1 year; operated upon	—	Yes‡	Yes	Normal	Normal
11	F	Living 5 years	—	Yes	Yes‡	Normal	Normal
12	F	Died 2 months	Yes	Yes‡	Yes	Mirror-image dextrocardia	Incomplete inversion
13	M	Living 3 years	—	Yes	Yes‡	Left-sided	Inverted
14	F	Died 28 years; operated upon‡	No	No	Yes	Normal	Normal
15	M	Living 2 years	—	Yes	Yes‡	Normal	Normal
16	F	Living	—	No	Yes‡	Normal	Normal

*IVC, inferior vena cava; RIVC, right inferior vena cava; RSVC, right superior vena cava; LIVC, left inferior vena cava; LSVC, left superior vena cava; LA, left atrium; RA, right atrium; SVC, superior vena cava.

†See text, classification of anomalous inferior vena cava with azygos continuation.

§Previously reported.[3]

‡Initial means of diagnosis.

when viewed in the lateral position. It is sometimes possible to advance the catheter tip from the azygos vein through the superior vena cava into the right atrium, usually by backing a loop into the heart, but further advancement of the catheter tip into the right ventricle and pulmonary artery is difficult. Confusion is even more likely to arise if the catheter tip passes via a hemiazygos vein into a left superior vena cava and thence into the coronary sinus and right atrium. These anomalous catheter positions have been illustrated by Loogen and Rippert.[15] Cardiac catheterization by way of the arm veins may be considered "normal," other than inability to advance the catheter tip far down the inferior vena cava (entrance into the hepatic veins may be misinterpreted as normal inferior vena cava), or passage into a large azygos or hemiazygos vein (Fig. 5).

It is important that this anomaly be known to the thoracic surgeon lest the azygos vein be sacrificed in the course of pulmonary or cardiac operation.[6, 7]

EMBRYOLOGIC AND ANATOMIC CONSIDERATIONS

Normal development. The inferior vena cava system has been the subject of innumerable studies, both because of its complex embryologic origin and because of its marked anatomic variability. The embryologic picture to be presented here is a deliberate

*IVC drainage and other systemic venous anomalies**	*Cardiac anomalies*	*Pattern†*
RIVC to azygos to RSVC	Cor biloculare with pulmonary atresia	A2
RIVC to azygos to RSVC	Cor biloculare with pulmonary stenosis	A1
LIVC to hemiazygos to LSVC to LA. Bilateral SVC	Probable cor biloculare	H6
RIVC to azygos to RSVC	Double outflow right ventricle; ventricular septal defect, PDA, right pulmonary veins to right atrium, left pulmonary veins to coronary sinus	A1
RIVC to hemiazygos to LSVC to coronary sinus to RA. Bilateral SVC	Ventricular septal defect, pulmonary stenosis, small left atrium and left ventricle	H1
RIVC to azygos to RSVC. Hypoplastic hepatic-prerenal segment of IVC	Probable cor biloculare	A6
RIVC to left-sided azygos to left-sided SVC	Ventricular and atrial septal defects, PDA, right pulmonary veins to left-sided atrium	A3
RIVC to azygos to RSVC	Single atrium with cleft mitral and tricuspid valve leaflets	A1
RIVC to azygos to RSVC	Probable cor biloculare, PDA	A1
RIVC to azygos to RSVC	Valvular pulmonary stenosis	A1
IVC to hemiazygos to LSVC to coronary sinus to RA. Bilateral SVC	Probable tricuspid atresia with pulmonary stenosis	H1
RIVC to right-sided hemiazygos to right-sided SVC to coronary sinus to left-sided systemic atrium. Bilateral SVC	Atrioventricular canal, subaortic stenosis, right-sided PDA. Left pulmonary veins to left-sided atrium	H3
RIVC to azygos to RSVC	Tricuspid atresia and transposition of great vessels	A6
IVC to azygos to RSVC	Atrial septal defect	A1
LIVC to hemiazygos to LSVC to coronary sinus to RA. Probable bilateral SVC	Ventricular septal defect	H1
RIVC to azygos to RSVC	Atrial septal defect	A1

simplification, and is not intended to indicate agreement in all details among the many anatomists and embryologists who have contributed to the literature on this subject. The primitive cardinal systems are complex, with a host of interconnecting channels. However, the diagram shown in Fig. 6 includes only those basic features needed for an understanding of the origin of the normal inferior vena cava and azygos vein and the apparent origin of the various anomalies under discussion. To facilitate interpretation, the various cardinal systems and their derivatives have been designated by separate number ranges (e.g., 20's for postcardinal, 30's for subcardinal). This diagram, and the following discussion, are based primarily on the work of McClure and Butler,[25] Gladstone,[26] and Seib,[19] and to a lesser extent on the reports of Reagan,[27] Butler,[28] and McClure and Huntington.[29]

Inferior vena cava. The inferior vena cava originates by the fusion and alteration of components of several different primitive venous pathways, and can thus be viewed as consisting of distinct segments.

Hepatic segment (pars hepaticae). The omphalomesenteric vein plexi fuse very early, draining by way of the two efferent vessels (venae revehentes hepatis) that join the sinus venosus (confluence of anterior and posterior cavae). Partial fusion of the two afferent vessels (the venae advehentes) contributes to the formation of the hepatic portal vein. Rightward displacement of the sinus venosus attachment to the heart leads to greater blood flow in the right vena revehens, and subsequent atrophy of the left vena revehens. Caudal extensions from the vena revehens or associated plexus join with the right subcardinal vein to establish continuity with the prerenal segment.

Table II. Cases from the literature

Author	*Date*	*Sex*	*Age*	*Autopsy*	*Operation*	*Special studies*		*Position of heart*
						Angiocardiography	*Cardiac catheterization*	
Miller[2]	1925	F	2 months	Yes‡	No	No	No	Left-sided
Gouley and Gilbert[3]	1939	F	9 days	Yes‡	No	No	No	Dextroversion(?)
Huseby and Boyden[4]	1941	F	Stillborn	Yes‡	No	No	No	—
Latimer and Virden[5]	1944	M	60 years	Yes‡	No	No	No	—
Taussig[6]	1947	F	25 years	Yes‡	No	No	No	Dextrocardia
Effler et al.[7]	1951	M	54 years	Yes	Yes‡ (Lung)	No	No	Normal
Campbell et al.[8]	1952	M	5 years	Yes	Yes	Yes	Yes‡	Left-sided
Stackelberg et al.[9]	1952	F	3 months	—	—	Yes‡	—	Normal
	1952	F	1 year	—	—	Yes‡	—	Normal
Downing[10]	1953	M	1 year	Yes	No	Yes‡	No	Left-sided
	1953	M	5 years	No	No	Yes	Yes‡	Normal
Levinson et al.[11]	1953	F	4 years	—	Yes	Yes‡	Yes	Normal
Campbell and Deuchar[12]	1954	M	6 years	Yes‡	Yes	—	—	Dextrocardia
	1954	M	6 weeks	Yes‡	—	—	—	Dextrocardia
	1954	F	6 years	—	—	Yes‡	Yes	Dextrocardia
Druepple[13]	1957	F	4 days	Yes†	No	No	No	Normal
Grover et al.[14]	1957	F	1 month	Yes	—	Yes†	No	Left-sided
Loogen and Rippert[15]	1958	M	18 years	No	Yes	—	Yes†	Normal
	1958	M	25 years	No	—	Yes	Yes†	Levoversion
	1958	M	—	—	—	Yes	Yes†	Normal
Schmutzer et al.[16]	1958	F	15 years	No	Yes	Yes	Yes†	Mirror-image dextrocardia
Kjellberg et al.[17]	1959	F	17 years	—	—	—	Yes†	Normal
	1959	M	9 years	—	—	—	Yes†	Normal

Position of abdominal viscera	IVC drainage and other systemic venous anomalies*	Cardiac anomalies	Pattern†
Partial inversion	IVC to azygos to RSVC to RA. Bilateral SVC	Biloculate heart and transposition of great vessels	A6
—	LIVC to hemiazygos to LA (LSVC?)	Ostium primum defect, PDA, coarctation of aorta	H2 (?)
—	Bilateral IVC to azygos to RSVC to RA	—	A1
Normal	Bilateral IVC to azygos to RSVC to RA	—	A1
Inverted	"Absent IVC"	Biloculate heart, pulmonary stenosis, anterior origin of aorta	—
Normal	LIVC to azygos to RSVC to RA	—	A1
Inverted	IVC to azygos to RSVC to right side of atrium. Bilateral SVC	Biloculate heart, pulmonary atresia, PDA	A6
Normal	RIVC to azygos to RSVC to RA	—	A1
—	IVC into hemiazygos (?) to LSVC to LA	Atrial septal defect plus	H1
Partial inversion	IVC to azygos directly to RA. No RSVC. LSVC directly into LA	Ventricular septal defect, pulmonary stenosis	A6
—	IVC to azygos to RSVC to RA	Ventricular septal defect	A1
Normal	RIVC to azygos to RSVC to RA	Total anomalous pulmonary venous connection to SVC and RA	A1
Inverted	IVC to left-sided azygos to LSVC to left-sided venous atrium. Bilateral SVC	Single atrium, ventricular septal defect, common A-V valve; aorta and pulmonary artery from anterior ventricle	A3
Normal	IVC to left-sided azygos to LSVC to left side of atrium	Single atrium, ventricular septal defect, common A-V valve; aorta and pulmonary artery from anterior ventricle	A4
Normal	IVC to left-sided azygos to LSVC to left-sided venous atrium	Intracardiac defects (cyanotic)	A4
Normal	RIVC to azygos to RSVC to RA. Bilateral SVC	Single atrium, PDA, aorta and pulmonary artery from right ventricle, pulmonary veins connect to portal vein	A1
Partial inversion	RIVC to azygos to RSVC to RA	Total anomalous pulmonary venous connection to right atrium	A6
Normal	RIVC to azygos to RSVC to RA	Atrial septal defect	A1
Partial inversion	IVC to left-sided azygos to left-sided SVC to left-sided venous atrium	Atrial septal defect	A5
Normal	IVC to azygos to RSVC to RA	Atrial septal defect	A1
Inverted	IVC to right-sided hemiazygos to coronary sinus to left-sided venous atrium. Bilateral SVC	Ventricular septal defect, PDA	A3
—	IVC to azygos to RSVC to RA	—	A1
—	IVC to hemiazygos to LSVC to coronary sinus to RA	—	H1

Table II. Cont'd

Author	*Date*	*Sex*	*Age*	*Autopsy*	*Operation*	*Special studies*		*Position of heart*
						Angiocardiography	*Cardiac catheterization*	
	1959	F	26 years	—	Yes	Yes	Yes†	Normal
Edwards[18]	1960	—	—	Yes†	—	—	—	Mirror-image dextrocardia

*See footnote to Table I.
†See text, classification of anomalous inferior vena cava with azygos continuation.
‡Initial means of diagnosis.

Prerenal segment (pars subcardinalis). Following the postcardinal veins in development, the two subcardinal plexi appear, joining the posterior cardinal veins anteriorly, and draining the mesonephri posteriorly. The subcardinal plexi form a midline anastomosis in the region of the mesonephroi, and the right subcardinal vein loses its anterior connection with the posterior cardinal vein, draining instead into the hepatic segment just described. The flow of blood through this channel increases, presumably because of flow predominance of the right vena revehens, and the anterior portion of the left subcardinal vein disappears except for the segment contributing to the left adrenal vein.

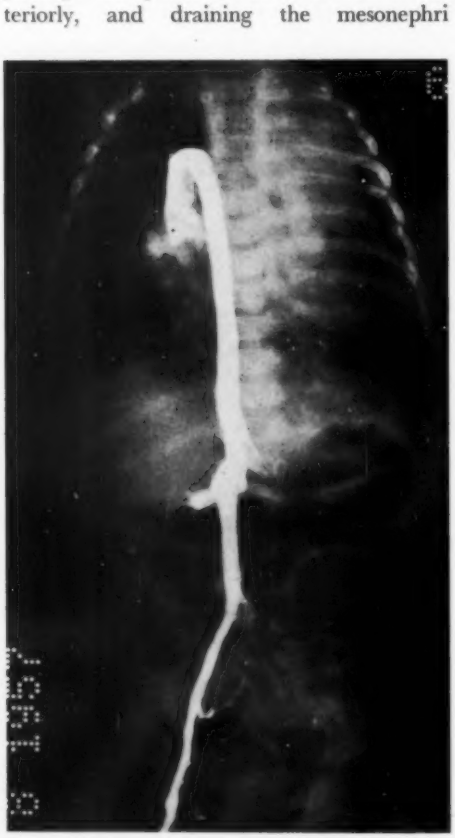

Fig. 1. Angiocardiogram, anteroposterior view, Case 7. Note passage of opaque material from right iliac vein to right postrenal inferior vena cava to azygos vein to right superior vena cava to right atrium.

Renal segment (pars renalis). Numerous anastomoses develop between the posterior cardinal, subcardinal, and supracardinal veins. The major anastomosis between the right subcardinal vein and the right supracardinal vein in the kidney region persists as the renal segment of the inferior vena cava.

Postrenal segment (pars supracardinalis). The supracardinal veins constitute the last set of major venous plexi to develop, and connect anteriorly and posteriorly with the posterior cardinal veins. That portion of the right supracardinal vein extending posteriorly from the subcardinal-supracardinal anastomosis (renal segment) to the postcardinal anastomosis persists as the postrenal segment of the inferior vena cava.

The azygos-hemiazygos system. The azygos and hemiazygos veins are derived from the anterior portions of the supracardinal veins, except for the short proximal arch that is postcardinal in origin. The intermediate portions of the supracardinal veins that connect with the subcardinal plexi in the kidney region (right and left lumbar

Position of abdominal viscera	*IVC drainage and other systemic venous anomalies**	*Cardiac anomalies*	*Pattern†*
—	IVC to azygos to RA	Partial anomalous pulmonary venous connection to azygos	A1
Inverted	IVC to right-sided hemiazygos to RSVC to coronary sinus to left-sided atrium. Bilateral SVC	Atrial septal defect, anomalous connection of left-sided pulmonary veins	H3

azygos veins[26]) remain small or disappear. The principal blood flow into the azygos and hemiazygos veins then comes from the the ascending lumbar veins, which represent longitudinal anastomoses of the lumbar intersegmental veins. The adult azygos vein is thus considered to originate in the thoracic cavity from a common vein usually formed by the junction of the right ascending lumbar vein with the right twelfth intercostal vein. The anterior connection of the hemiazygos vein is generally lost, and this vein then drains across into the azygos vein.

Disagreement exists as to the continuity of the supracardinal system as described. Thus, Reagan[27] and Seib[19] consider the azygos vein to represent a venous line different from the one producing the postrenal segment of the inferior vena cava. The reader is referred to Seib for amplification of this theory, which, despite its complexity, actually appears to offer a better explanation of anatomic variation.

Anatomic considerations. Depending upon the persistence of the various individual cardinal vein components shown in Fig. 6, as well as various anastomotic channels not shown, an unlimited number of major and minor variants in final structure are possible.[29] Seib[19] lists 63 types of postrenal inferior venae cavae alone, and, from his own data and those of others, estimates the general incidence of bilateral postrenal inferior vena cava to be 3 per cent, and of left inferior vena cava alone, 0.5 per cent. Our clinical experience suggests that the incidence of left postrenal inferior vena cava is considerably higher than this in patients with congenital heart defects.

In his classification of anomalies of the inferior vena cava, Edwards[30] lists anomalous inferior vena cava with azygos continuation as the only major anomaly of the prerenal segment of this vein. However, variation in the azygos-hemiazygos system of veins is especially great, both in regard to their lumbar roots and to the final makeup

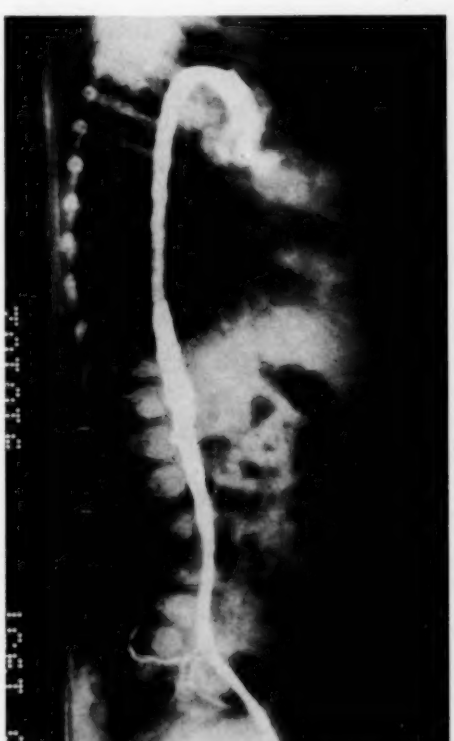

Fig. 2. Angiocardiogram, lateral view, Case 7. Note "candy cane" appearance of anomalous venous pathway.

Fig. 3. Angiocardiogram, anteroposterior view, Case 3. Opaque material passes from left inferior vena cava to hemiazygos vein to left superior vena cava to left atrium.

of the hemiazygos vein. Thus, Seib[19] described 23 different patterns in his study of 200 adult cadavers.

The inferior vena cava and azygos systems normally function as largely independent drainage systems, yet possess numerous anastomotic channels that permit alternate pathways of blood flow when obstruction to flow occurs in either system. These connections, as well as the connections with the vertebral system of veins, have been best demonstrated in recent years by angiocardiography.[31, 32] Much of this work has been initiated as a result of interest in venous obstructive lesions[33] and in the vascular spread of malignancies.[34]

In routine angiocardiography performed from the leg veins, the degree of opacification of collateral channels varies considerably, and is probably unrelated to the nature of the cardiac defects that may be present. In addition to opacification of the inferior vena cava, there is usually a greater or lesser degree of opacification of the ascending lumbar veins by way of the iliac anastomotic veins or lumbar veins. The lattice-like vertebral venous plexi are then opacified by way of the lumbar and intercostal veins. The ascending lumbar veins may be identified well up into the thoracic cavity, where they communicate with the azygos and hemiazygos veins through the intercostal veins. This opacification of the azygos system is slow, and is ordinarily not seen until 3 to 6 seconds after injection of the opaque material in the malleolar vein in infants and small children.

Anomalous inferior vena cava with azygos continuation. This anomaly is by no means a uniform one, but consists of a series of venous variants. Basically, there are 2 types, those with azygos continuation and those with hemiazygos continuation (Figs. 1 and 3). With the hope that correlations might be found with specific cardiac defects, a further classification was devised on the basis of the position of the viscera and of the heart. The cases in Tables I and II were classified in this manner, and the totals are listed in parentheses as follows:

A. Azygos continuation (32)
   1. Normal position of heart and abdominal viscera (18)
   2. Dextroversion of heart (1)
   3. Mirror-image dextrocardia with transposed abdominal viscera (4)
   4. Mirror-image dextrocardia without transposed abdominal viscera (2)
   5. Situs inversus with levoversion: mirror-image of dextroversion of the heart (1)
   6. Complete or incomplete transposition of abdominal viscera alone (6)

H. Hemiazygos continuation (8)
   1. Normal position of heart and abdominal viscera (5)
   2. Dextroversion of heart (1?)
   3. Mirror-image dextrocardia with transposed abdominal viscera (2)
   4. Mirror-image dextrocardia without transposed abdominal viscera (0)
   5. Situs inversus with levoversion: mirror-image of dextroversion of the heart (0)
   6. Complete or incomplete transposition of abdominal viscera alone (0)

It is readily apparent that the common pattern is azygos continuation with normal position of the heart and abdominal viscera (type A-1). There was no evident relationship of these groupings with the type of cardiac defect present. As can be seen in Tables I and II, bilateral superior venae cavae typically occur when there is hemiazygos continuation, but are common also with azygos continuation. Thus, the incidence of left superior vena cava is considerably higher than the 3 to 10 per cent figure cited earlier for persistent left superior vena cava among all patients with cardiac defect. Much less commonly there may be an absence of the superior vena cava on the side where the azygos or hemiazygos vein drains.[10, 17] Hemiazygos and azygos veins may both be of large size prior to the drainage of the former into the latter (Fig. 7), and may be associated with bilateral postrenal inferior venae cavae. The hemiazygos vein may drain directly into the left atrium rather than through the coronary sinus into the right atrium (Fig. 3). Rarely, a hypoplastic prerenal segment may be identified (Fig. 8), or both the prerenal segment and the azygos vein may be of large size.[29]

Fig. 4. Angiocardiogram, anteroposterior view, Case 12. Situs inversus with mirror-image dextrocardia. Note passage of opaque material from right inferior vena cava to right-sided hemiazygos vein to right-sided "left superior vena cava" to coronary sinus to left-sided venous atrium. Slight opacification of ascending lumbar vein and vertebral plexus is also evident.

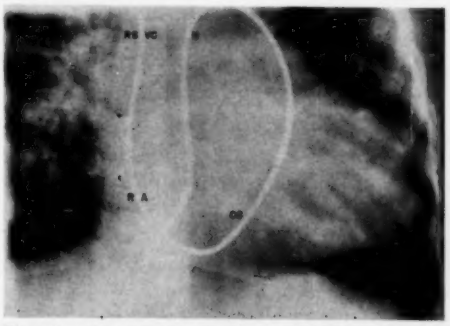

Fig. 5. Roentgenogram of chest, posteroanterior view, Case 15. Cardiac catheter passes from right superior vena cava *(RSVC)* to right atrium *(RA)* to coronary sinus *(CS)* to left superior vena cava *(RSVC)* to hemiazygos vein *(H)*.

It is of interest that Patients 7 and 12 and the patient of Grover and associates had multiple spleens, as well as partial inversion of the abdominal viscera. No instances of absent spleen were noted.

This anomaly must be distinguished from suprahepatic obliteration or obstruction of the inferior vena cava,[35] which is probably secondary to disease, and from connection of a normal inferior vena cava (that is, with hepatic segment present) with the left atrium.[36]

The embryologic basis of anomalous inferior vena cava with azygos continuation is readily apparent in Fig. 6. Failure of union of the hepatic *(64)* and subcardinal *(33)* segments necessitates drainage of the blood by the lumbar and thoracic azygos and/or hemiazygos systems. Either the right lumbar azygos segment *(43)* that connects *(75)* with the dorsal aspect of the inferior vena cava in the renal region or the corresponding left (hemiazygos) segment *(44)* that connects *(76)* with the left renal vein or dorsal aspect

of the inferior vena cava then persists as the main trunk. Both roots may persist as large vessels in some cases (Fig. 7).

Campbell and Deuchar[12] found their 4 cases to be associated with transposed abdominal viscera, and suggested that the contralateral development of upper and lower inferior vena cava tends to prevent normal fusion. The association of this anomaly with abnormally placed abdominal viscera in the other cases reviewed is frequent but is not the usual finding. Common factors apparently underlie both the cardiac and venous anomalies, often with disturbances in the transition from symmetrical to asymmetrical vascular patterns. Early differences in preferential blood flow are very likely involved.

TERMINOLOGY

Anomalous inferior vena cava with azygos continuation has been described under a variety of terms: absent inferior vena cava, anomalous inferior vena cava with azygos drainage, absence of the hepatic segment of the inferior vena cava, absence of the prerenal segment of the inferior vena cava, persistence of the supracardinal vein, persistence of the subcardinal vein, and continuation of the postcardinal vein. It is obvious that none of these terms adequately describes and explains the anomaly, and some are clearly false. The addition of further terms would probably add to the confusion, though it would seem more informative to describe the anomaly as "infrahepatic interruption of the inferior vena cava with azygos (hemiazygos) continuation," recognizing at the same time that there may have been an embryologic failure of fusion rather than a secondary interruption. In addition, identification of the position of the postrenal inferior vena cava (right, left, or bilateral) in these cases would be helpful. As already emphasized, the lower inferior vena cava segment often normally connects directly with both the hepatic segment and the azygos vein, but with the azygos connection (lumbar azygos) being only 1 to 3 mm. in diam-

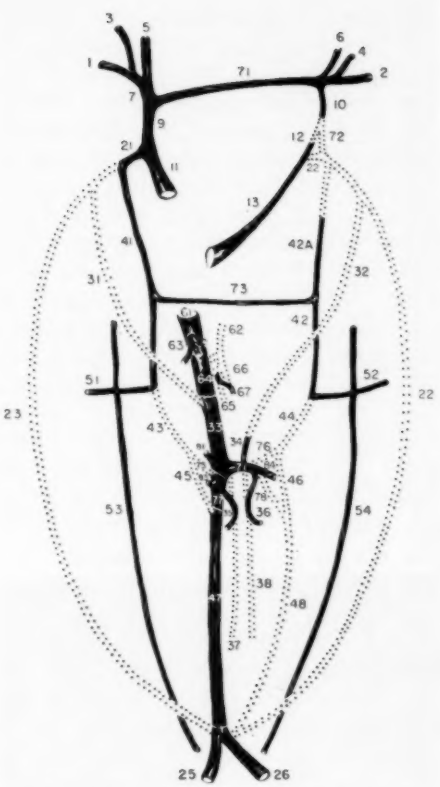

Fig. 6. Schematic representation of primitive venous systems and the final adult vessels derived from them. Final structures are shown in solid lines; temporary venous channels are shown in broken lines.

Anterior and common cardinal system

1. Right subclavian vein
2. Left subclavian vein
3. Right external jugular vein
4. Left external jugular vein
5. Right internal jugular vein
6. Left internal jugular vein
7. Right innominate vein
9. Right superior vena cava (anterior cardinal)
10. Left superior intercostal vein
11. Right superior vena cava (common cardinal)
12. Oblique vein of Marshall
13. Coronary sinus (common cardinal)

Posterior cardinal system

21. Terminal arch of azygos
22. Left postcardinal vein
23. Right postcardinal vein
25. Right iliac vein
26. Left iliac vein

Subcardinal system

31. Right subcardinal vein
32. Left subcardinal vein
33. Prerenal segment of inferior vena cava
34. Left adrenal vein
35. Right gonadal vein (portion from subcardinal)
36. Left gonadal vein (portion from subcardinal)
37. Right subcardinal vein
38. Left subcardinal vein

Supracardinal system

41. Azygos vein
42. Hemiazygos vein
42A. Accessory hemiazygos vein
43. Lumbar azygos vein (often persists in adult). Derived from supracardinal vein and minor anastomosis *(75)* between this vein and subcardinal vein
44. Lumbar hemiazygos vein (may persist in adult). Derived from supracardinal vein and minor anastomosis *(76)* between this vein and subcardinal vein
45. Right supracardinal vein
46. Left supracardinal vein
47. Postrenal segment of inferior vena cava
48. Left supracardinal vein (may persist as left postrenal inferior vena cava)

Lumbar and intercostal system

51. Right twelfth intercostal vein
52. Left twelfth intercostal vein
53. Right ascending lumbar vein
54. Left ascending lumbar vein

Omphalomesenteric system

61. Hepatic segment of inferior vena cava (right vena revehens)
62. Left vena revehens
63. Hepatic veins
64. Hepatic segment of inferior vena cava (caudal extension)
65. Right vena advehens
66. Left vena advehens
67. Portal vein (venae advehentes)

Anastomotic channels and miscellaneous veins

71. (black). Left innominate vein (anastomosis between anterior cardinals)
71. (white). Anastomosis between subcardinals (forms part of left renal vein)
72. Anastomosis between left anterior and posterior cardinals
73. Anterior anastomosis between supracardinals
75. Minor anastomosis between right subcardinal and supracardinal
76. Minor anastomosis between left subcardinal and supracardinal
77. Renal segment of inferior vena cava (major anastomosis between right subcardinal and supracardinal
78. Major anastomosis between left subcardinal and supracardinal
81. Right adrenal vein
83. Right renal vein
84. Left renal vein

Makeup of adult inferior vena cava

Hepatic segment *(61, 64)*
Prerenal segment *(33)*
Renal segment *(77)*
Postrenal segment *(47)*

Makeup of anomalous inferior vena cava with azygos or hemiazygos continuation

Interruption or failure of fusion of hepatic *(64)* and prerenal *(33)* segments, and persistence of right lumbar azygos vein *(43)* or left lumbar hemiazygos vein *(44)*

Fig. 7. Angiocardiogram, anteroposterior view, Case 9. Opaque material passes from right inferior vena cava to azygos vein and hemiazygos vein (by way of left renal vein) and then to right superior vena cava and right atrium. Left ascending lumbar vein is also opacified.

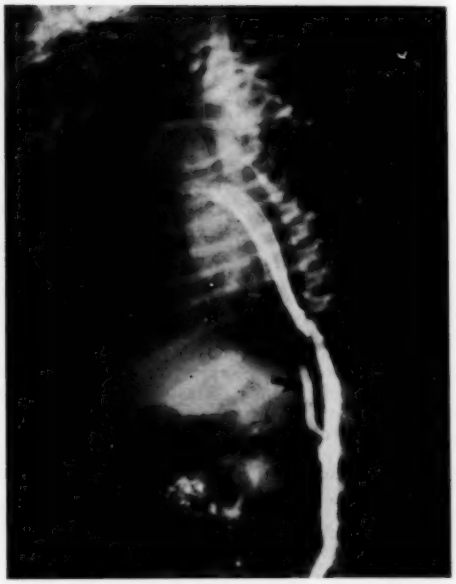

Fig. 8. Angiocardiogram, right posterior oblique view, Case 6. Note that main drainage is by way of azygos vein, but that hypoplastic prerenal segment of inferior vena cava is also present (indicated by arrow).

eter.[19] Thus the boundary between normal and abnormal anatomy in this area is not as great as appears superficially.

SUMMARY

Fifteen new cases of anomalous inferior vena cava with azygos or hemiazygos continuation are presented, together with a review of 26 cases previously published. The incidence of this anomaly approximates 0.6 per cent in patients with congenital heart defects. The commonly encountered cardiac defects include cor biloculare, atrioventricular canal, anomalously connecting pulmonary veins, double outflow right ventricle, large atrial septal defect, pulmonary stenosis or atresia, or combinations of these. Abnormal positions of the heart and abdominal viscera are often present. An antemortem diagnosis can be established by cardiac catheterization or angiocardiography. A typical "candy cane" appearance of the anomalous venous pathway occurs when angiocardiography is performed from the leg veins. The most common pattern is azygos continuation with normal position of the heart and abdominal viscera. There is no evident relationship between the subtypes of this anomaly and the intracardiac defects that may be present.

The embryologic development of the venous system is discussed, particularly in regard to the origin of the anomalies under discussion. Anomalous inferior vena cava with azygos or hemiazygos continuation is best interpreted as interruption or failure of fusion of the hepatic and prerenal segments of the inferior vena cava, combined with persistence of either the right lumbar azygos vein or left lumbar hemiazygos vein. Thus, a better term for the anomaly would be "infrahepatic interruption of the inferior vena cava with azygos (hemiazygos) continuation."

REFERENCES

1. Anderson, R. C., Heilig, W., Novick, R., and Jarvis, C.: Anomalous Inferior Vena Cava With Azygous Drainage: So-called Absence of the Inferior Vena Cava, Am. Heart J. **49:** 318, 1955.
2. Miller, A. J.: Congenital Heart Disease With Partial Situs Inversus, Absence of the Inferior Vena Cava, and Other Anomalies, Am. J. Path. **1:** 467, 1925.
3. Gouley, B. A., and Gilbert, P. D.: Left Inferior Vena Cava, Arch. Path. **27:** 1021, 1939.
4. Huseby, R. A., and Boyden, E. A.: Absence of the Hepatic Portion of the Inferior Vena Cava With Bilateral Retention of the Supracardinal System, Anat. Rec. **81:** 537, 1941.
5. Latimer, H. B., and Virden, H. H.: A Case of Complete Absence of the Inferior Vena Cava, J. Kansas M. Soc. **45:** 346, 1944.
6. Taussig, H. B.: Congenital Malformations of the Heart, New York, 1947, The Commonwealth Fund, p. 510.
7. Effler, D. B., Greer, A. E., and Sifers, E. C.: Anomaly of the Vena Cava Inferior, J. A. M. A. **146:** 1321, 1951.
8. Campbell, M., Gardner, F., and Reynolds, G.: Cor Biloculare, Brit. Heart J. **14:** 317, 1952.
9. Stackelberg, B., Lind, J., and Wegelius, C.: Absence of Inferior Vena Cava Diagnosed by Angiocardiography, Cardiologia **21:** 583, 1952.
10. Downing, D. F.: Absence of the Inferior Vena Cava, Pediatrics **12:** 675, 1953.
11. Levinson, D., Griffith, G. C., Cosby, R. S., Zinn, W. J., Jacobson, G., Dimitroff, S. P., and Oblath, R. W.: Transposed Pulmonary Veins, Am. J. Med. **15:** 143, 1953.
12. Campbell, M., and Deuchar, D. C.: The Left-sided Superior Vena Cava, Brit. Heart J. **16:** 423, 1954.
13. Druepple, L.: Complete Pulmonary Venous Drainage Into the Portal Vein With Multiple Congenital Anomalies, Am. Heart J. **53:** 790, 1957.
14. Grover, R. F., Lanier, R. R., Lowry, J. K., and Blont, S. G.: Congenital Absence of the Hepatic Portion of the Inferior Vena Cava, Am. Heart J. **54:** 794, 1957.
15. Loogen, F., and Rippert, R.: Anomalien der grossen Körper- und Lungenvenen, Ztschr. Kreislaufforsch. **47:** 677, 1958.
16. Schmutzer, K. J., and Linde, L. M.: Situs Inversus Totalis Associated With Complex Cardiovascular Anomalies, Am. Heart J. **56:** 761, 1958.
17. Kjellberg, S. R., Mannheimer, E., Rudhe, U., and Jonsson, B.: Diagnosis of Congenital Heart Disease, ed. 2, Chicago, 1959, The Year Book Publishers, Inc., p. 12.
18. Edwards, J. E.: Malformations of the Thoracic Veins, in Gould, S. E.: Pathology of the Heart, ed. 2, Springfield, Ill., 1960, Charles C Thomas, Publisher, p. 483.
19. Seib, G. A.: The Azygos System of Veins in American Whites and American Negroes, Including Observations on the Inferior Caval Venous System, Am. J. Phys. Anthropol. **19:** 39, 1934.
20. Papez, J. W.: Two Cases of Persistent Left Superior Vena Cava in Man, Anat. Rec. **70:** 191, 1938.
21. Sanders, J. M.: Bilateral Superior Vena Cava, Anat. Rec. **94:** 657, 1946.
22. Adachi, B.: Statistik der Varietäten der V. cava caudalis bei den Japanern, Anat. Anz. **85:** 215, 1937.
23. Dwight, T.: Absence of the Inferior Cava Below the Diaphragm, J. Anat. & Physiol. **35:** 7, 1901.
24. Stauffer, H. M., LaBree, J., and Adams, F.: The Normally Situated Arch of the Azygos Vein: Its Roentgenologic Identification and Catheterization, Am. J. Roentgenol. **66:** 353, 1951.
25. McClure, C. F. W., and Butler, E. G.: The Mammalian Inferior Vena Cava, Am. J. Anat. **35:** 331, 1925.
26. Gladstone, R. J.: Development of the Inferior Vena Cava in the Light of Recent Research, With Especial Reference to Certain Abnormalities, and Current Descriptions of the Ascending Lumbar and Azygos Veins, J. Anat. **64:** 70, 1929.
27. Reagan, F. P.: A Century of Study Upon the Development of the Eutherian Vena Cava Inferior, Quart. Rev. Biol. **4:** 179, 1929.
28. Butler, E. G.: The Relative Role Played by the Embryonic Veins in the Development of the Mammalian Vena Cava Posterior, Am. J. Anat. **39:** 267, 1927.
29. McClure, C. F. W., and Huntington, G. S.: The Mammalian Vena Cava Posterior, Am. Anat. Mem. No. 15, Philadelphia, 1929, Wistar Institute of Anatomy and Biology.
30. Edwards, E. A.: Clinical Anatomy of Lesser Variations of the Inferior Vena Cava; and a Proposal for Classifying the Anomalies of This Vessel, Angiology **2:** 85, 1951.
31. Abrams, H. L.: The Vertebral and Azygous Venous Systems, and Some Variations in Systemic Venous Return, Radiology **69:** 508, 1957.
32. Abrams, H. L.: The Relationship of Systemic Venous Anomalies to the Paravertebral Veins, Am. J. Roentgenol. **80:** 414, 1958.
33. Mellins, H. Z.: Functions of the Vertebral Venous System, Bull. Univ. Minnesota Hosp. **22:** 213, 1951.
34. Batson, O. V.: The Function of the Vertebral Veins and Their Role in the Spread of Metastases, Ann. Surg. **112:** 138, 1940.
35. Denie, J. J. P. A., and Verheugt, A. P. M.: Un cas d'oblitération de la partie supra-hépatique de la veine cave inférieure, Acta cardiol. **15:** 78, 1960.
36. Gardner, D. L., and Cole, L.: Long Survival With Inferior Vena Cava Draining Into Left Atrium, Brit. Heart J. **17:** 93, 1955.

Repetitive paroxysmal auricular tachycardia with defective intraventricular conduction in a 7-month-old infant

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REPETITIVE or intermittent paroxysmal tachycardia was reported for the first time by Lewis[5] in 1909, when, with the aid of the polygraph, he recorded 54 paroxysms of auricular tachycardia within 109 minutes in an otherwise normal individual. The ventricular form was described independently for the first time by Gallavardin[1-3] in France and by Scott[1] in England in 1922. The subject was reviewed in detail by Parkinson and Papp[6] in 1947, who recommended the term repetitive paroxysmal tachycardia for the first time. Since that time, many cases of this type have figured in the literature and have been commented upon favorably, in the matter of prognosis, by most authors.

In view of certain very unusual features, both clinical and electrocardiographic, the following case report is considered worthy of publication.

CASE RECORD

A Hindu male infant, aged 7 months and weighing 16 pounds, was admitted to the Bombay Hospital on Aug. 15, 1960, for diarrhea, vomiting, convulsions, and restlessness. Apart from diarrhea of 2 days' duration, the patient was in perfect health until 2 P.M. on the day of admission, when, during a phase of restful sleep, he woke up suddenly with vomiting and restlessness and became cold and clammy.

On examination, the child, otherwise well nourished and normally built, was in obvious distress with a markedly rapid and irregular, uncountable pulse, and a temperature of 100° F. The blood pressure was not recordable. On auscultation, a "tic-tac" rhythm was heard over the heart, without murmurs. No signs of congestive heart failure were present and the other systems of the body appeared normal. The patient was given continuous oxygen and 2 c.c. paraldehyde and 50 mg. tetracycline intramuscularly.

An electrocardiogram (Fig. 1) taken at 9 P.M., on the same day, showed an apparently ventricular form of repetitive paroxysmal tachycardia, large, bizarre ventricular complexes occurring in groups of 6 to 10 beats at the rate of from 260 to 300 per minute and separated by short pauses of about 0.30

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second, without intervening sinus or ectopic beats. Because of the contour of the complexes, the irregularity of the ventricular beats, and absence of all evidence of auricular activity, the condition was regarded as ventricular in origin, that is, until the time of the second electrocardiogram (Fig. 2). An initial dose of 300 mg. of hydroquinidine, was given, followed by injection of 75 mg. every 6 hours.

A second electrocardiogram (Fig. 2) taken 3 hours later showed bizarre ventricular complexes occurring in groups and separated by short pauses as in Fig. 1, but with several important differences: (1) The runs of beats tended to be longer. (2) The rate of beating was far more constant and slower, being fixed at 180 beats per minute. (3) Evidences of normal auricular activity (normal P waves with P-R intervals of 0.12 second) were present in the form of single P waves at the onset of each paroxysm. (4) Evidences of abnormal or ectopic auricular activity were observed in the form of small, upright, and peaked P′ waves interposed clearly between the successive T waves and QRS complexes of each paroxysm in Lead II, with P′R intervals of 0.05 second. (5) Regular notching of the bizarre waves of each paroxysm, through the presence of P′ waves, was also discernible on careful inspection of the other leads of the tracing. The condition was clearly one of repetitive auricular paroxysmal tachycardia, with aberrant ventricular conduction resulting in notching and increased duration of QRS complexes. The absence of normal sinus beats and of ectopic or extrasystolic beats during the pauses and the presence of a single P wave of normal type prior to each paroxysm were other features of unusual interest in this tracing. On the basis of this tracing (taken within 3 hours of the first), it became necessary to regard the first cardiogram (Fig. 1) also as an example of atypical repetitive auricular paroxysmal tachycardia. Because of the rapid rate (260 to 300 per minute) and gross degree of aberration of ventricular complexes in Fig. 1, detection of signs of auricular activity, in the form of P′ waves, was apparently not possible. Irregular ventricular beating as in Fig. 1, although rare in supraventricular paroxysmal tachycardia, can and does occur in cases where the runs of beats are short, the arrhythmia in such cases being variously ascribed to (1) irregular discharge of the ectopic pacemaker, (2) presence of more than one ectopic pacemaker, or (3) dispersion of the paroxysmal tachycardia among sinus beats.[4] The grossly bizarre appearance of the ventricular beats in Fig. 1 could be explained on the grounds of a severe aberration of ventricular conduction. In view of persisting restlessness and vomiting, the child received an injection of 10 mg. chlorpromazine, and became quiet within a few hours.

A third electrocardiogram (Fig. 3) taken at 10:30 A.M. on Aug. 16, 1960, showed a pattern similar to that of Fig. 2, the rate and contour of the QRS complexes being identical, but with two major differences: The tachycardia was no longer repetitive but continuous, no intermediate pauses being detectable, and evidence of auricular activity in the form of P′ waves in Lead II was absent. Closer inspection of the tracing, how-

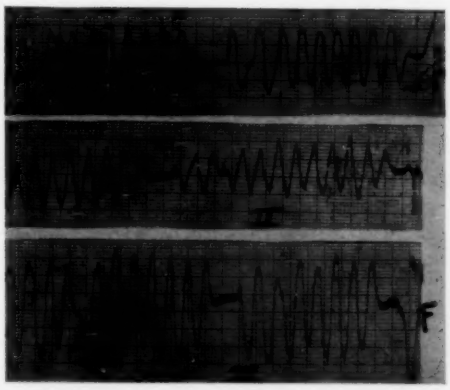

Fig. 1. Electrocardiogram on admission (9 P.M. on Aug. 15, 1960) shows repetitive paroxysmal tachycardia, with groups of 6 to 10 bizarre waves separated by short pauses. Regarded at first as ventricular in origin, because of configuration of waves, absence of P waves, and mild arrhythmia (rate 260 to 300 per minute), but later ascribed to an auricular origin, after availability of the second cardiogram (Fig. 2).

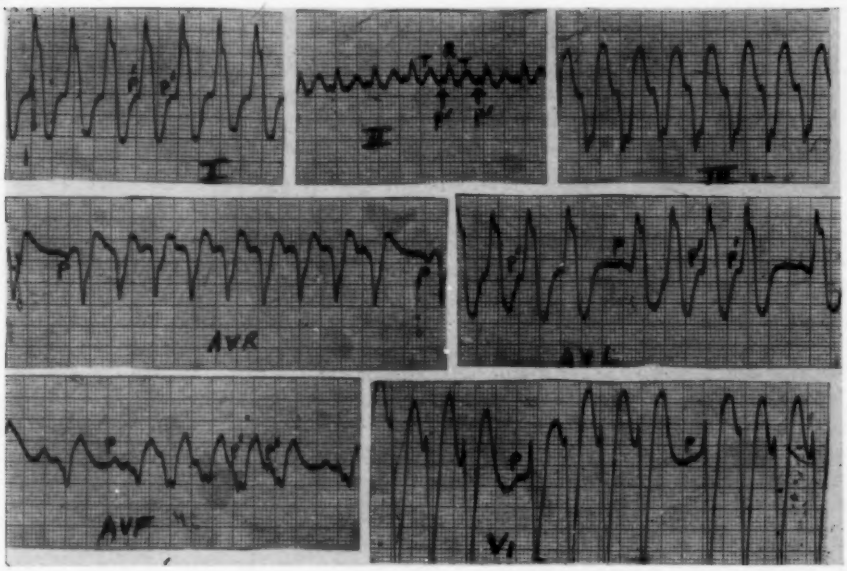

Fig. 2. Electrocardiogram, 3 hours later, shows paroxysms of bizarre QRS complexes with a single normal P wave at the end of each pause, and P′ waves of ectopic origin between each pair of T waves and QRS complexes in Lead II and as "notches" in the other leads. Rate of beating, 180 per minute. Example of repetitive paroxysmal tachycardia of auricular type with aberrant ventricular conduction.

ever, suggested a fusion of the P′ and T waves, as the result of increased duration of the Q-T interval (from 0.31 to 0.36 second) and appearance of a well-defined S-T segment.

A fourth electrocardiogram (Fig. 4) taken at 8:40 P.M on Aug. 16, 1960, showed restoration of normal sinus rhythm, without evidence of ectopic beating or aberrant ventricular conduction. The cardiac rate was 126 per minute; normal and upright P waves were clearly visible with P-R intervals of 0.12 second, and the QRS duration was 0.06 second. The characteristic feature of this tracing (also observed in subsequent tracings over a period of several days) was a marked depression of the S-T segment with T wave inversion in some of the leads, particularly in leads V_1, V_2, III, and aVF. The S-T-T abnormalities were ascribed to a quinidine effect upon the so-called posttachycardial syndrome (usually ascribed to transitory coronary insufficiency or myocardial ischemia through rapid heart action).

With restoration of normal sinus rhythm, the child continued to improve clinically, until the morning of Aug. 17, 1960, when he was found to be dyspneic and coughing. Although the heart rate was still about 130 per minute, the lungs were full of râles and rhonchi, the liver was enlarged (one finger-breadth), and the abdomen tympanitic With restricted fluid intake, oral administration of dichlorothiazide, 12.5 mg. daily, and 0.20 mg. daily of digoxin (purified glycoside of *Digitalis lanata*), in 3 equally divided doses, the child made a rapid clinical recovery. He was discharged from the hospital, 3 days later in a normal condition with a pulse rate of 110 per minute, and no evidence of cardiac decompensation. Fig. 5 represents the final electrocardiogram taken at 10:30 A.M. on Aug. 19, 1960, at the time of discharge. Despite the normal sinus rhythm and S-T-T abnormalities of a minor order which were present, clinically and radiologically, the heart size appeared normal.

COMMENT

The case described here presents several features of unusual interest, both clinical and electrocardiographic. The repetitive form of paroxysmal tachycardia, although possible at any age, is distinctly unusual during infancy. The high rate of ectopic beating, as recorded during the initial paroxysms (rate of 300 per minute as in Fig. 1) is also uncommon, although rates of over 300 (even 345 per minute on one occasion) have been reported occasionally. The development of congestive cardiac failure, through repetitive paroxysmal tachycardia in a normal infant, with no evidence of cardiovascular disease, is of considerable clinical interest.

Electrocardiographically, the case is of even greater interest, presenting as it does a unique sequence of electrocardiographic events within the matter of only 24 hours. Fig. 1 illustrates the difficulty encountered at times in differentiating a paroxysmal ventricular tachycardia from a paroxysmal supraventricular tachycardia with aberrant

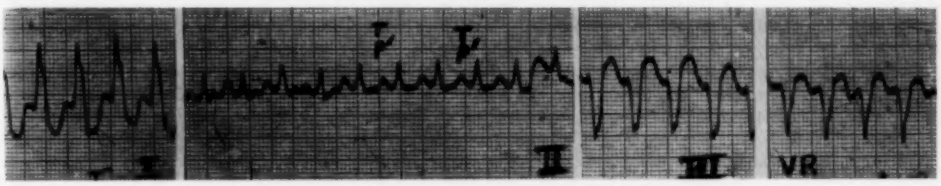

Fig. 3. Electrocardiogram (10:30 A.M. on Aug. 16, 1960) shows same pattern and rate as in Fig. 2, but with no pauses, the tachycardia being continuous. Evidence of auricular activity in Lead II, absent because of increased duration of Q-T interval resulting in fusion of P′ and T waves.

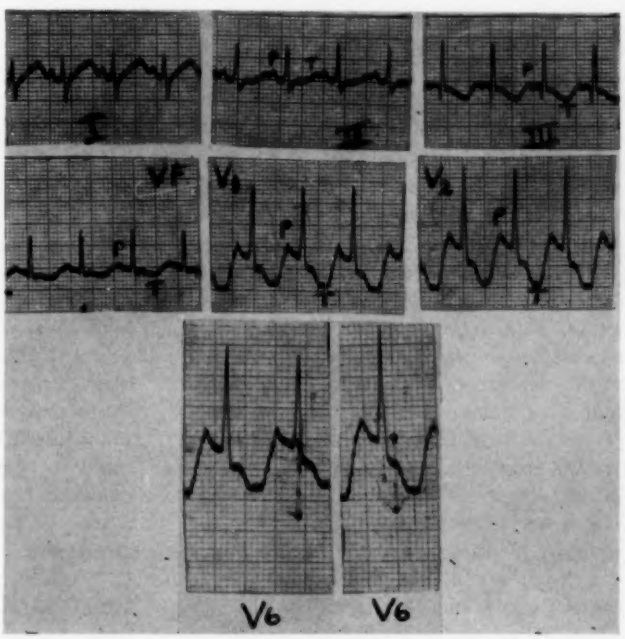

Fig. 4. Electrocardiogram (8:40 P.M. on Aug. 16, 1960) shows restoration of normal sinus rhythm with normal P wave activity, normal QRS complexes, P-R interval of 0.12 second, and grossly abnormal S-T-T segments (particularly in leads CR_1 and CR_2) probably secondary to a combination of quinidine effect and posttachycardial syndrome. Later electrocardiograms (during the next few days) showed identical S-T-T patterns.

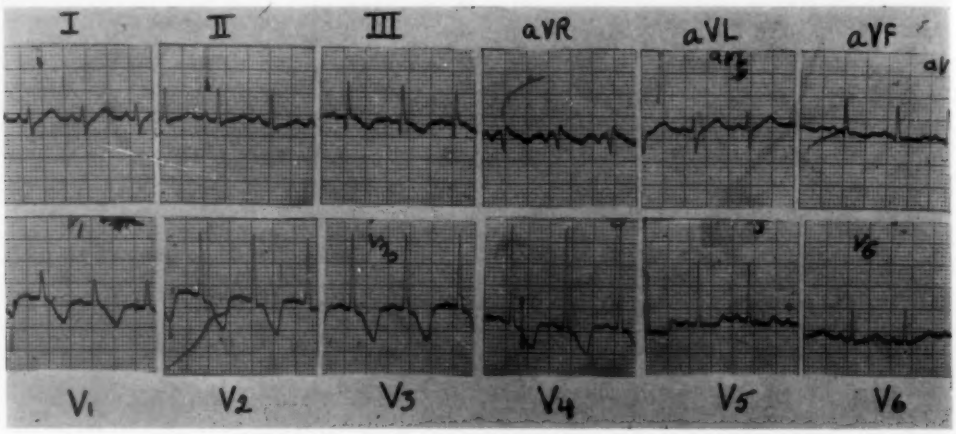

Fig. 5. Complete electrocardiogram of patient, taken at the time of discharge, showing restoration to normal sinus rhythm, with a mild degree of sinus tachycardia and depression of S-T-T segments in leads V_1 to V_3 (see text).

ventricular conduction. The first electrocardiogram (Fig. 1) was regarded as an example of ventricular tachycardia until proved otherwise by a second electrocardiogram (Fig. 2) recorded 3 hours later. Fig. 1 presents many other points of interest, viz., the irregular beating of the ventricles, the bizarre appearance of the ventricular complexes, the high rate of beating, absence of normal or ectopic beating during the intermediary pauses, and absence of all discernible evidence of auricular activity in the tracing.

Fig. 2, besides revealing the true nature of the ectopic rhythm or cardiac arrhythmia, shows slowing of the ectopic rhythm from about 280 to 180 per minute through quinidine administration within the matter of about 3 hours. It also displays evidence of normal auricular activity in the form of a normal P wave and P-R interval at the onset of each paroxysm and of abnormal or ectopic auricular activity in the form of P′ waves or upright peaks between the T waves and QRS complexes of Lead II and as indistinct notches in the other leads. Another feature of interest about this tracing is the regularity of discharge of the ectopic center at a fixed rate of 180 per minute, unlike that of the previous electrocardiogram.

Fig. 3, although superficially similar to Fig. 2, shows absence of P′ waves (of ectopic auricular activity) in Lead II, because of an increase in duration of the Q-T interval from 0.31 to 0.36 second, resulting in fusion of P′ and T waves. Also, the ectopic rhythm has lost its repetitive character.

Fig. 4, besides showing a restoration of normal P waves and QRS complexes, displays characteristic alterations of S-T segments and T waves, through association of quinidine effect with a posttachycardial syndrome. Further electrocardiographic tracings, taken on subsequent days, showed persistence of these S-T-T abnormalities.

SUMMARY

Repetitive paroxysmal tachycardia of auricular type with aberrant intraventricular conduction which simulated paroxysmal ventricular tachycardia is reported in an infant 7 months of age. Among the noteworthy features about the case were: (1) the bizarre ventricular complexes, (2) high and variable rate of discharge (up to 300 per minute) of the ectopic focus, (3) lack of evidence of ectopic auricular activity, (4) absence of sinus or extrasystolic beats between paroxysms, (5) increase in duration of the Q-T interval, and (6) development of persistent S-T-T segment abnormalities with restoration of normal sinus rhythm.

REFERENCES

1. Gallavardin, L.: De la tachycardie paroxystique à centre excitable, Arch. mal. coeur. **15:** 1, 1922.
2. Gallavardin, L.: Extrasystolie ventriculaire à paroxysmes tachycardiques prolongés, Arch. mal. coeur. **15:** 298, 1922.
3. Gallavardin, L.: Extrasystolie auriculaire à paroxysmes tachycardiques, Arch. mal. coeur. **15:** 774, 1922.
4. Katz, L. N., and Pick, A.: A Clinical Electrocardiography. Part I. The Arrhythmias With an Atlas of Electrocardiograms, Philadelphia, 1956, Lea & Febiger.
5. Lewis, T.: The Mechanism and Graphic Registration of the Heart Beat, ed. 3, London, 1925, Shaw and Sons.
6. Parkinson, J., and Papp, C.: Repetitive Paroxysmal Tachycardia, Brit. Heart J. **9:** 241, 1947.
7. Scott, R. W.: Heart **9:** 224, 1921-1922 (quoted by Parkinson and Papp[6]).

Punctate epiphyseal dysplasia, another cause of leg length inequality

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PUNCTATE epiphyseal dysplasia is a relatively rare disorder of infancy involving cartilaginous epiphyses and apophyses. The disease was described first by Conradi[1] in 1914. Several reports have appeared since, but the relative rarity of the condition is evidenced by the fact that the largest series reported by an individual author[2] was 4 cases. A recent review[3] listed 55 cases in the world literature.

The disease has been described under a number of synonyms including stippled epiphyses, chondrodystrophia calcificans congenita, Conradi's disease, chondrodystrophia congenita punctata, and others. The number of names serves also to indicate the paucity of knowledge about the disease.

The characteristic histologic change is the focal deposition of lime in masses of degenerating connective tissue among the growing cartilages of both primary and secondary ossification centers.[4] Roentgenologically, the epiphyses present a stippled appearance, as if ossifying from many different centers. This appearance has been likened to that produced by flicking paint from a brush onto a clean surface.[5] These calcifications within the epiphyses vary in size and number, but tend to be multiple and minute. There is a definite tendency for regression to occur with increasing age; and if the patient survives, the epiphysis eventually appears normal. However, as Phillips[6] has emphasized, the epiphysis does not arise from fusion of the discrete calcifications, as these are abnormal deposits which gradually disappear.

The epiphyses most frequently involved are those of the upper and lower ends of the femur, upper end of the tibia, and upper end of the humerus. The lower end of the tibia and both ends of the fibula, radius, and ulna are much less frequently affected. Apparently, any cartilaginous epiphysis can be involved.

Clinically, the disease can be divided into 2 forms, on the basis of degree of involvement and prognosis. The first is a severe form with generalized epiphyseal involvement. It is often associated with multiple skeletal abnormalities, dwarfing, cataracts, cutaneous abnormalities, low birth weight, and often terminates in death by the age of 2 years. Cataracts, in particular, usually indicate a poor prognosis.[3] Death commonly occurs as a result of overwhelming renal or pulmonary infection.

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Table I. Scanogram comparison of bone length (in centimeters) in lower extremities

Bone	*Right*	*Left*	*Difference*
Femur	34.5	34.3	-0.2
Tibia	29.4	26.2	-3.2
Total	63.9	60.5	-3.4

A less severe form also occurs in which involvement may be limited to single epiphysis or restricted to one extremity and may or may not be associated with the previously mentioned abnormalities. The prognosis in this form is good, although a residual bony deformity may result.

No biochemical abnormalities have been detected in this disease. A definite familial tendency has been noted, the most likely mode of transmission being Mendelian recessive. Both sexes have been affected about equally.

In reviewing the world literature, we have been able to find only 3 cases that have been followed past the age of 4½ years. Selakovich and White[7] reported a case with monomelic involvement which was followed for 11 years, and eventually displayed shortening and hypoplasia of the involved extremity. Fairbank[5] mentioned, but did not describe in detail, a case with monomelic involvement that was followed for 9 years and showed residual leg shortening. Paul[8] reported a case with generalized involvement of epiphyses without associated abnormalities which he followed for 9 years; here, too, there was residual shortening of an involved lower leg.

We have had the opportunity recently to observe the result of this disease process after a period of 8 years and 3 months, and have seen the residual deformity which has resulted from an otherwise benign instance of this poorly understood disease.

REPORT OF A CASE

An 8-year-old white girl was first seen at the Mayo Clinic in May, 1960, because of unequal length of the legs. She was the product of a full-term, uncomplicated pregnancy and delivery, and had appeared normal in every respect at birth. At 5 weeks of age she had had pneumonitis and had been hospitalized in another institution. After a chest x-ray had accidentally included the left distal femoral epiphysis, further x-rays of the inferior extremities had been obtained which revealed the typical changes of punctate epiphyseal dysplasia (stippled epiphyses) involving the distal femoral epiphysis, proximal and distal tibial epiphyses, and proximal metatarsal structures of the left inferior extremity only (Fig. 1). The pneumonitis had responded well to antibiotic therapy, but repeat films of the left

Fig. 1. Both legs at age 5 weeks. Multiple minute calcifications in region of distal femoral epiphysis and proximal and distal tibial epiphyses of left leg. Left distal femoral epiphysis somewhat smaller than right. Calcifications in metatarsal area are not visible in this view.

leg at 9 weeks of age had been essentially unchanged (Fig. 2).

The patient's growth and development had proceeded normally, and she was reported to have walked at 11 months of age. However, at about the time walking began it had been observed that the left leg was somewhat shorter than the right. Repeat films at the age of 25 months had shown no evidence of stippling, and the previously involved epiphyses had appeared normal (Fig. 3). Follow-up films taken at age 6½ years had shown normal development of the distal femoral, proximal tibial, and proximal fibular epiphyses (Fig. 4). The left leg had remained shorter than the right, and a built-up shoe had been worn. Despite this, it had been noted that scoliosis was developing in the dorsal-lumbar spine, and the patient had been referred to the Mayo Clinic for further study.

Fig. 2. Left leg at age 9 weeks, essentially unchanged from Fig. 1. Calcifications in metatarsal area are plainly visible.

Fig. 3. Left leg at age 25 months. Complete disappearance of previous calcifications and normal epiphyseal development.

The patient was the fifth of 9 siblings. Four siblings were said to have had infantile eczema, and a fifth sibling had asthma. There was no family history of inequality of leg length, of congenital abnormalities, or of parental consanguinity.

On physical examination the patient was rated in the seventy-fifth percentile in height and weight. No cataract formation was seen on funduscopic examination. The left foot appeared mildly hypoplastic and the left tibia and fibula were shortened with resultant pelvic tilt and compensatory dorsal-lumbar scoliosis. No other abnormalities were present.

Scanograms for bone length of the lower extremities revealed that the right tibia was 3.2 cm. longer than the left (Table I). There was also evidence of minimal osteochondrosis of the medial tibial condyle.

From Green and Anderson's tables[9] it appeared that the remaining potential for tibial growth (in length) was approximately 4.5 cm. Because the shortening was confined to the left tibia and fibula, stapling of the right tibia and fibula was recommended to forestall further lengthening on that side. On June 23 the right tibial epiphysis was stapled with 6 stainless-steel staples, and the right fibular epiphysis with 1. The procedure was tolerated well and the patient returned home on the seventh postoperative day. Follow-up 1 month later revealed a normal

Fig. 4. Left leg at age 6½ years. Epiphyseal development proceeding normally.

range of motion at the knee joint; and the patient was swimming, skating, and riding her bicycle.

COMMENT

Since diagnosis is dependent upon roentgenologic examination, it appears likely that this case of punctate epiphyseal dysplasia would not have been diagnosed had it not been for the episode of pneumonitis at age 5 weeks when the disease was discovered accidentally. The epiphyseal changes in this condition are usually resolved by age 2 years,[3, 6] and residual deformities may not yet be apparent at this time. Hence, at least some of the instances of localized deformity and hypoplasia of the skeletal system that are seen in later life may well be due to this condition, which is no longer recognizable in the older child or adult. If the typical associated abnormalities are present, it may be possible to arrive at the correct diagnosis even though the pathognomonic epiphyseal changes have long since disappeared.

It seems evident that with recognition of the disorder and properly timed corrective surgery it may be possible to ameliorate many of the residual deformities.

SUMMARY

A case of punctate epiphyseal dysplasia which was followed for 8 years and 3 months is presented. The course of the disease in this instance was relatively benign, but the patient displayed residual shortening of the involved extremity, for which surgical therapy was instituted. This entity can be added to the list of causes of inequality of leg length. Since the distinctive signs may disappear before the inequality of development becomes evident, this disease may be the cause of such inequality more often than is realized.

REFERENCES

1. Conradi, Erich: Vorzeitiges Auftreten von Knochen- und eigenartigen Verkalkungskernen bei Chondrodystrophia fötalis hypoplastica: Histologische und Röntgenuntersuchungen, Jahrb. Kinderh., Leipz. **80:** 86, 1914.
2. Raap, G.: Chondrodystrophia Calcificans Congenita, Am. J. Roentgenol. **49:** 77, 1943.
3. Allansmith, Mathea, and Senz, Edward: Chondrodystrophia Congenita Punctata (Conradi's Disease), Am. J. Dis. Child. **100:** 109, 1960.
4. Caffey, John: Pediatric X-ray Diagnosis: A Textbook for Students and Practitioners of Pediatrics, Surgery, and Radiology, ed. 3, Chicago, 1956, The Year Book Publishers, Inc., p. 777.
5. Fairbank, H. A. T.: An Atlas of General Affections of the Skeleton. 8. Dysplasia Epiphysialis Punctata: Synonyms—Stippled Epiphyses, Chondrodystrophia Calcificans Congenita (Hünermann), J. Bone & Joint Surg. **31-B:** 114, 1949.
6. Phillips, L. I.: Chondrodystrophia Calcificans Congenita, New Zealand M. J. **56:** 22, 1957.
7. Selakovich, W. G., and White, J. W.: Chondrodystrophia Calcificans Congenita: Report of a Case, J. Bone & Joint Surg. **37-A:** 1271, 1955.
8. Paul, L. W.: Punctate Epiphyseal Dysplasia (Chondrodystrophia Calcificans Congenita): Report of Case With Nine Year Period of Observation, Am. J. Roentgenol. **71:** 941, 1954.
9. Green, W. T., and Anderson, M.: Experiences With Epiphyseal Arrest in Correcting Discrepancies in Length of Lower Extremities in Infantile Paralysis: Method of Predicting the Effect, J. Bone & Joint Surg. **29:** 659, 1947.

Congenital megacolon (Hirschsprung's disease) associated with hypoproteinemia and edema

A case report

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THE diagnosis and treatment of congenital megacolon (Hirschsprung's disease) in infancy are well established.[1-3] An examination of the available medical literature fails to mention the association of edema and hypoproteinemia in a young infant with congenital megacolon.

CASE REPORT

J. R. G., a white male infant who weighed 7 pounds was born in another hospital on Jan. 6, 1958. The parents were healthy and there was no history of familial or contagious disease. This was the first pregnancy of the 17-year-old mother and was described as uneventful. The infant was delivered spontaneously from a vertex presentation after a 9-hour labor. The immediate neonatal course was uneventful. Between the fifth and seventh days of life the infant's stools became excessive and he was changed from breast feeding to a Similac formula. By 10 days of age the number of stools had decreased and they ceased to be spontaneous. The abdomen became distended; he began vomiting; this was followed by diarrhea and dehydration. He was admitted to another hospital and given fluids parenterally and penicillin and streptomycin intramuscularly. Between the age of 2 weeks and 1 month, the baby had many episodes of alternating diarrhea and constipation. I first saw him when he was 1 month of age. He had developed abdominal distention associated with projectile vomiting over a 24-hour period, and had not passed any stools during this time.

Physical examination. At the time of admission the baby weighed 6 pounds, 13¾ ounces; his height was 20¼ inches. The temperature, rectally, was 98.6° F. The abdomen was distended and he was in moderately acute distress. He was thin and emaciated; the skin was smooth and moist with a minimum of subcutaneous fat. A mass was found in the left lower quadrant of the abdomen on deep palpation. This was thought to be feces in the sigmoid colon. The liver and spleen were not enlarged.

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Digital examination of the rectum discovered no feces for at least 3 inches. There was 2 plus pitting edema of the dorsal surfaces of the hands and feet and of the pretibial areas.

Laboratory studies. Examination of the blood showed a hemoglobin level of 12.8 Gm. per cent and a white blood count of 26,000 per cubic millimeter, with 4 per cent myelocytes, 2 per cent juvenile cells, 4 per cent stab cells, 14 per cent segmented cells, 53 per cent lymphocytes, and 21 per cent monocytes. Analysis of a voided urine specimen showed a slight trace of albumin and 2 to 4 white blood cells per high-power field. The results of two other urinalyses were similar. Other blood studies showed a total serum protein of 3.8 Gm. per cent (2.4 Gm. per cent albumin and 1.4 Gm. per cent globulin); blood urea nitrogen, 25 mg. per cent; serum chloride, 94 mEq. per liter; serum sodium, 149 mEq. per liter; thymol turbidity, 4.1 units; and total cholesterol, 85 mg. per cent. The coagulation time was 4 minutes, 16 seconds, and the bleeding time 3 minutes, 55 seconds. The cephalin flocculation test was reported to be negative at 48 hours. A blood culture was sterile. An x-ray of the chest failed to show anything abnormal. When given a barium enema, the sigmoid colon appeared redundant. No narrowing of the distal rectum was visible. Barium was present in the sigmoid colon 48 hours after the barium enema was given. An intravenous pyelogram showed no abnormalities.

Course. The physical examination and x-ray findings suggested the diagnosis of congenital megacolon. A biopsy of the rectal mucosa was made. No ganglion cells were noted in either the intermuscular or the submucosal plexuses. An exploratory laparotomy was performed through a left paramedian incision. The sigmoid colon was found to be large, measuring 3 to 4 cm. in diameter. The rectum was small distally measuring 1½ cm. in diameter. A sigmoid colostomy of the simple loop type was done to bring the dilated segment to the outside. A biopsy of the liver was taken at the time of operation. The infant withstood the operation well. The postoperative course was uneventful, and he was discharged on the seventh day. At this time no edema of the hands and feet was noted. One month after the sigmoid colostomy the total serum protein was 7.4 Gm. per cent with 4.6 Gm. per cent albumin, and 2.8 Gm. per cent globulin. Electrophoresis of these serum proteins revealed a normal pattern. At 18 months of age a Swenson pull-through operation was performed without difficulty. The infant has done well postoperatively. He has normal daily bowel movements without difficulty.

Pathological reports. A specimen from the dilated area of the colon showed the following: Ganglion cells were abundant within the muscular plexus. The serosa was edematous and focally contained small numbers of fibroblasts and histiocytes. The overlying mucosa was generally intact although there were focal areas of chronic inflammation consisting of large numbers of histiocytes within the mucosa and extending into the submucosa.

In a specimen from the narrow segment of the rectum, ganglion cells were completely absent from the intermuscular and submucosal plexus. The mucosa was normally formed with no evidence of inflammation.

A report of a biopsy of the liver described the architecture as well preserved and the parenchymal cells intact. The individual cells were finely vacuolated, apparently because of deposits of glycogen. The Kupffer cells contained granules of pigment, presumably hemosiderin. Small focal areas of extramedullary hematopoiesis were encountered.

DISCUSSION

A 1-month-old infant with congenital megacolon was seen because of alternating constipation and diarrhea associated with peripheral edema. Subsequent studies revealed a marked hypoproteinemia. Loss of protein through the urinary tract was excluded by failure to find excessive protein in

the urine. Liver disease was ruled out by the results of the liver function tests and biopsy of the liver.

It is believed that the hypoproteinemia in this case was secondary to the loss of protein into the lumen of the colon during periods of alternating diarrhea and fecal impaction. The phenomenon of protein loss into the bowel lumen during chronic diarrhea is well established.[4, 5] In this patient, the serum proteins returned to normal levels after a colostomy controlled the fecal impaction and secondary diarrhea. The possibility that this infant did not have his normal quota of serum protein at birth cannot be excluded.

SUMMARY

The case of a 1-month-old infant with congenital megacolon (Hirschsprung's disease), edema, and hypoproteinemia is presented. The possible etiology of these unusual findings is discussed. It is suggested that the serum protein was lost through the bowel lumen.

I wish to express my appreciation to Dr. Harvey Rosenberg, Pathologist of The Texas Children's Hospital, Houston, Texas, for the pathological reports quoted, to Dr. Louis E. Gibson who performed the colostomy, and to Dr. Theodore P. Votteler who carried out the second-stage procedure.

REFERENCES

1. Swenson, O., Newhauser, E. B. D., and Pickett, L. K.: New Concepts of Congenital Megacolon, Pediatrics **4:** 201, 1949.
2. Nelson, W. E.: Textbook of Pediatrics, ed. 7, Philadelphia, 1959, W. B. Saunders Company, p. 673.
3. Gross, R. E.: The Surgery of Infancy and Childhood, Philadelphia, 1953, W. B. Saunders Company, p. 330.
4. Ulstrom, R. A., and Krivit, W.: Exudative Enteropathy, Hypoproteinemia, Edema, and Iron-Deficiency Anemia, A. M. A. J. Dis. Child. **100:** 509, 1960.
5. Nelson, W. E.: Textbook of Pediatrics, ed. 7, Philadelphia, 1959, W. B. Saunders Company, p. 357.

Cerebrospinal fluid otorrhea and recurrent meningitis

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IN FREQUENTLY recurring otitic meningitis resulting from a fracture of the skull, the route of infection may be difficult to detect.[1-3] This is a report of an unusual case of a child in whom otitic meningitis recurred 20 times, requiring the combined efforts of the pediatric, radiologic, otolaryngologic, and neurosurgical departments of the Children's Hospital of Philadelphia and 4 surgical procedures before the route of infection was finally discovered and obliterated. From a review of this and similar cases,[1-6] a method of clinical approach is suggested for the investigation of recurrent otitic meningitis.

REPORT OF A CASE

This boy had a total of 29 admissions to the Children's Hospital of Philadelphia. He was first admitted in 1948 at 17 months of age with meningitis and bilateral mastoiditis (Table I). The meningitis responded well to antibiotic therapy, after which a bilateral simple mastoidectomy was performed. There was no history of trauma to the skull and x-ray examination showed no evidence of any fracture of the skull.

During the next year and a half the child had 6 episodes of meningitis. Following the sixth episode, indigo carmine was injected into the cerebrospinal fluid, but no communication between the ear or nose and the cerebrospinal fluid could be demonstrated.

Multiple readmissions for meningitis ensued, all of which were preceded by upper respiratory infections, and on 9 occasions by otitis media. Nine years after the first admission the child was admitted for the sixteenth episode of meningitis. Following recovery, a re-exploration of the original mastoid cavity on the right side was done, and, again, nothing unusual was noted by the surgeon. On the eighteenth admission injection of dye into the cerebrospinal fluid established the presence of cerebrospinal fluid rhinorrhea from the right side. Following another bout with meningitis, it became apparent that the child also had cerebrospinal fluid otorrhea in the right ear.

The otorrhea was confirmed by injecting

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Table I. Summary of admissions and bacteriologic findings

Admission	*Diagnosis*	*Throat culture*	*Nasopharyngeal culture*	*Cerebrospinal fluid culture*	*Culture of ear or mastoid*
1	Meningitis	Pneumococcus *H. influenzae*	Pneumococcus *H. influenzae*	Pneumococcus	Pneumococcus
2	Meningitis	*N. catarrhalis* Pneumococcus	Staphylococcus	*H. influenzae*	Staphylococcus
3	Pharyngitis	*N. catarrhalis* Pneumococcus			
4	Meningitis	*N. catarrhalis* Pneumococcus	Staphylococcus Pneumococcus	Meningococcus	Negative
5	Tonsillectomy and adenoidectomy				
6	Meningitis	Staphylococcus *N. catarrhalis*	Staphylococcus	Pneumococcus	
7	Otitis media		*N. catarrhalis* Pneumococcus		
	Pharyngitis	No growth	Streptococcus		
8	Meningitis	*N. catarrhalis* Pneumococcus Streptococcus	Pneumococcus *N. catarrhalis*	Pneumococcus	
9	Otitis media	Pneumococcus *N. catarrhalis* Streptococcus	Staphylococcus Pneumococcus		
10	Pharyngitis	*N. catarrhalis* Pneumococcus	No growth	No growth	
11	Meningitis		Staphylococcus Pneumococcus	No growth	
12	Meningitis	*N. catarrhalis* Pneumococcus Diphtheroids	Staphylcoccus *N. catarrhalis*	No growth	
13	Otitis media	Yeast *N. catarrhalis* *H. influenzae* Pneumococcus	Staphylcoccus	No growth	

indigo carmine into the cerebrospinal fluid and noting the leakage of dye into the right middle ear and out of the nose. On a subsequent admission, Pantopaque was injected into the cisterna magna, and on x-ray examination there appeared to be some dye passing out of the right Eustachian tube. On neurosurgical exploration, closure of a suspected defect on the posterior surface of the right temporal bone was attempted with nylon mesh. One month later the child was readmitted with meningitis. Laminagrams in the reverse Towne position showed a linear fracture line extending into the lateral part of the petrous pyramid.

The right mastoid was then re-explored via a radical mastoidectomy. During the operation, dye was injected into the right ventricle and was seen to leak from the roof of the bony part of the right Eustachian tube in the area where the fracture line was visualized on x-ray examination. The bony opening of the right Eustachian tube was then packed with bone wax through the middle ear. Following this procedure there has been no further cerebrospinal fluid

Table I. Cont'd

Admission	*Diagnosis*	*Throat culture*	*Nasopharyngeal culture*	*Cerebrospinal fluid culture*	*Culture of ear or mastoid*
14	Meningitis	*N. catarrhalis*	Staphylococcus Pneumococcus Diphtheroids	Pneumococcus	
15	Meningitis		Pneumococcus Staphylococcus	Pneumococcus	
16	Gastroenteritis			No growth	
17	Meningitis		Staphylococcus Streptococcus	Pneumococcus	
18	Meningitis	Coli-aerogenes *H. influenzae* Pneumococcus	Staphylococcus *H. influenzae*	*H. influenzae*	
19	Meningitis		Staphylococcus *H. influenzae*	*H. influenzae*	
20	Meningitis	Enterococcus *H. streptococcus*		No growth	
21	Meningitis		Staphylococcus Diphtheroids	No growth	
22	Meningitis			No growth	
23	Meningitis		Staphylococcus *N. catarrhalis*	No culture	
24	Mastoid revision		Streptococcus		
25	Meningitis		Staphylococcus	No culture	
26	Meningitis	Staphylococcus Enterococcus		No culture	
27	Meningitis	Staphylococcus Streptococcus *N. catarrhalis*	*H. influenzae* Staphylococcus	*H. influenzae*	
28	Craniotomy				
29	Meningitis	Pneumococcus Staphylococcus Enterococcus	Pneumococcus Streptococcus Staphylococcus *N. catarrhalis*	No growth	No growth

otorrhea, and no further meningitis has occurred during a period of 2 years.

DISCUSSION

The most common cause of cerebrospinal fluid otorrhea is fracture of the skull.[6-9] The incidence of cerebrospinal fluid otorrhea in all of these fractures may be as high as 20 per cent.[7, 10, 11] The course of a fracture through the temporal bone is subject to considerable variation; the transverse type of fracture is the most dangerous. This usually involves the bony labyrinth, an area of ossification in cartilage (endochondral bone) which is, by its nature, predisposed to fibrous union.[1, 12, 13] This may constitute a persistent route of infection from the middle ear to the cerebrospinal fluid.[1-3, 14] However, any temporal bone fracture may result in a fibrous union with dimpling of the dura into the fracture line which prevents osseous union.

There are certain rare instances in which a fracture is not the cause of recurring meningitis. Cawthorne[5] mentioned a child with 18 episodes of otitic meningitis in which the *locus minoris resistentiae* was a persistent

petrosquamous suture. Another route of infection may be the natural arachnoidal extensions into the internal auditory meatus.[3]

Evidence of fracture is always to be sought, and the fracture line may be most difficult to detect on x-ray examination.[2, 4, 7] There is no one x-ray view that will best show fractures of the temporal bone.[15]

The presence of cerebrospinal fluid otorrhea may not be easy to establish. The spinal fluid may be masked by pus or blood. Sodium iodide can be injected into the subarachnoid space, and the presence of iodine in the middle ear can be established within one-half hour after the injection. A similar procedure is the use of indigo carmine injected into the cerebrospinal fluid. A radiopaque substance can also be instilled and the aid of the x-ray enlisted in tracing the path of otorrhea.

In the 10 instances of meningitis in which an organisms was isolated from the cerebrospinal fluid, a pneumococcus was isolated 6 times, *Hemophilus influenzae* 3 times, and the meningococcus once. In 6 instances the organism isolated from the cerebrospinal fluid was also found in the nasopharynx or throat.

Since the route of infection was via the Eustachian tube, one would expect a closer correlation between the cultures from the spinal fluid and the nose and throat.

Several studies have shown that cultures from the upper respiratory tract have little value in predicting the etiological agent of otitis media.[16, 17]

CONCLUSIONS

For the definitive therapy of each episode of meningitis, it is most important that cultures of the cerebrospinal fluid be obtained. In this case of cerebrospinal fluid otorrhea, bacteriologic studies of the upper respiratory tract were of little value in delineating the therapy of a complicating meningitis.

Laminagraphy is an essential study in searching for a possible fracture as the path of cerebrospinal fluid otorrhea.

If the site of the leak into the ear is not determined preoperatively, a radical mastoidectomy is essential to visualize the entire roof of the middle ear and the Eustachian tube. Dye should be injected into the cerebrospinal fluid at the time of operation.

SUMMARY

This is the report of an unusual case of a child in whom otitic meningitis recurred twenty times. Four surgical procedures were required before the route of infection was finally detected and obliterated.

Bacteriologic studies of the pathogens in the upper respiratory tract did not necessarily show correlation with organisms isolated on the culture of the cerebrospinal fluid.

Laminagraphy is essential in searching for a possible fracture of the skull as the path of cerebrospinal fluid otorrhea. If the site of leak into the ear is not determined preoperatively, a radical mastoidectomy is essential to visualize the entire middle ear and the Eustachian tube. Dye should be injected into the cerebrospinal fluid at the time of operation.

REFERENCES

1. Maxwell, H.: Recurrent Otogenic Meningitis, Laryngoscope **63:** 355, 1953.
2. Hara, A. J.: Otitic Meningitis in the Antibiotic Era, Ann. Otol., Rhin. & Laryng. **68:** 305, 1959.
3. Precechtel, A.: The Problem of Recurrent Meningitis in ORL, Acta oto-laryng. **45:** 427, 1954.
4. Muermann, O.: Acta oto-laryng. **45:** 429, 1954.
5. Cawthorne, T. E.: Acta oto-laryng. **45:** 429, 1954.
6. Dandy, W. E.: Treatment of Rhinorrhea and Otorrhea, Arch. Surg. **49:** 75, 1944.
7. Grove, W. E.: Otoneurology of Head Injuries, *in* Coates, G. M., Schenck, H. P., and Miller, H. V.: Otolaryngology, Hagerstown, 1959, W. F. Prior Company, Inc.
8. Skolnick, E. M., and Ferrer, J. L.: Cerebrospinal Fluid Otorrhea, Arch. Oto-laryng. **70:** 795, 1959.
9. Grove, W. E.: Otologic Symptoms or Complications of Craniocerebral Injury, Surg. Gynec. & Obst. **74:** 581, 1942.
10. Besley, F.: A Contribution to the Study of Skull Fractures, J. A. M. A. **66:** 345, 1932.
11. Phelps, C.: An Analytical and Statistical Review of 1000 Cases of Head Injury, Am. Surgeon 449, 1902.

12. Voss, O.: Der Chirurgie der Schädelbasisfrakturen, Leipzig, 1936, Erhfarhungen.
13. Grove, W. E.: Skull Fractures Involving the Ear, Laryngoscope **49:** 673, 1939.
14. Nager, F. R.: Late Meningitis Following Fracture of the Labyrinth, *in* Fowler's Loose Leaf Medicine of the Ear, New York, 1947, Thos. Nelson & Sons.
15. Shambaugh, G. E., Jr.: Surgery of the Ear, Philadelphia, 1950, W. B. Saunders Company.
16. Mortimer, E. A., Jr., and Watterson, R. L.: A Bacteriologic Investigation of Otitis Media in Infancy, Pediatrics **17:** 359, 1956.
17. Jones, M.: Comparison of Bacteria From Ear and Upper Respiratory Tract, Arch. Otolaryng. **72:** 329, 1960.

A study of relationships between family situation, bronchial asthma, and personal adjustment in children

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COMPREHENSIVE bibliographic surveys on the interrelationships between emotions and bronchial asthma are provided by Dunbar[6] and Leigh.[12] In general the literature on psychological factors in childhood asthma tends to reflect more conjecture than disciplined research, and there are many contradictions between reports. A number of major emphases in the literature can be delineated. Various attempts have been made to define a specific personality profile in asthmatic children. These efforts evolved at a time when discrete personality constellations were being sought in each psychosomatic illness.[7, 8] With the subsequent difficulties in confirming differences in pattern between the various diseases, there is today less stress on personality profile, although generalizations tend to linger. The asthmatic child, presumably every child with asthma, is thus described as markedly overanxious, demonstrating considerable latent aggression, egocentric, brighter than average, unusually orderly and clean, unable to tolerate frustration, oversensitive, overdemanding, and inhibited in the expression of feeling.[1, 5, 21, 22]

Another stress has been on specific aspects of the mother-child relationship as directly etiological. It has been suggested that the asthmatic attack is caused by fear of separation from the mother, and that it represents a suppressed cry for the mother.[10, 11] There has been speculation as to the mechanism through which the matrix or anlage for later asthma is established through specific distortions in the earliest mothering experience.[4] Some workers have suggested that maternal rejection is virtually universal, along with inhibition of opportunity for the expression of hostility toward the rejecting parent.[14-16] The role of a traumatic incident as trigger for an asthmatic attack has been described.[10, 12] In addition to the threat of separation from the mother, factors implicated are sudden strong emotions, physical overexertion, situations that provoke anger

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that cannot be expressed, fear of failure, and sexual conflicts of many types. The observation that separation from home has led in certain cases to marked improvement or total relief from asthma has encouraged the delineation of an "asthmatogenic climate" in the production of attacks.[2, 18-20]

As part of a general interest in psychosomatic problems, the psychiatrists in the present research team began, some 10 years ago, to collect data on asthmatic children whom they studied in psychiatric clinics and hospitals. Certain patterns of relationship between asthma and personality were suggested and generalizations implied, but as we reviewed our experience it became clear that we were not dealing with the general problem of asthma in children. Our patients in the psychiatric services were much too specialized to be considered representative of the total asthmatic child population. In general we were seeing two highly selected groups of cases:

1. Children with severe, sometimes intractable, asthma that had failed to respond to traditional medical management. It was customary, and still tends to be, to reserve these "unsuccessful" cases for the psychiatrist and to refer them as a last resort. It is evident that whatever findings emerge from the study of this highly selected group cannot be applied to all asthmatic children.

2. Children with severe behavior or adjustment problems who also suffered with asthma constituted the second group. In this group the asthma was coincidental; the patients would have been referred to the psychiatrist regardless of the presence of asthma. Again, generalization of findings from this group of disturbed children to all asthmatic children is clearly unwarranted.

It was evident that if we were to study the relationship between psychological factors and asthma we must use an unselected patient group, drawn not from the psychiatrists' but from the allergists' practice. In 1957 such a project was instituted. Allergists working in a university hospital clinic and in private practice joined with child psychiatrists to collaborate in the study of an unselected group of asthmatic children and their families. The children, undergoing hyposensitization for chronic asthma, formed the regular patient group of the allergists. The only selective criterion was that the families would be willing to cooperate in the study; with a few exceptions those who were invited agreed to participate and maintained cooperation.

THE PRESENT STUDY: HYPOTHESIS

We were interested in investigating three main aspects of the general problem: (1) the relationship between the family situation and the severity of asthma and response to medical management; (2) the extent to which specific psychological and experiential precipitants of attacks can be delineated; and (3) the effect of separation from the family on the course of asthma.

This paper reports the findings of our investigations into the first of these questions. The approach combined clinical and statistical research methods. Recognizing that disturbed family and parent-child relationships were inherent in the types of psychological correlates to childhood asthma, suggested in the literature and in our own previous cases, we began with the following hypothesis:

A study of asthmatic children and their families will reveal a positive relationship between the asthma situation (severity, course, response to treatment) and the family situation (quality of family relationships, identification opportunities, and care and handling).

As a control a second hypothesis was proposed: *A study of the same group of children and their families will reveal a positive relationship between level of disturbance in the child's personality and/or behavior and level of disturbance in family relationships and care and handling.*

ALLERGY INVESTIGATIONS

Seventy-one children and their parents were studied; all the patients were undergoing perennial hyposensitization treatment

Table I. Age on entering study

Years	*No.*
0 to 3	1
4 to 7	30
8 to 11	29
12 to 15	11
Total	71

Table II. Age at onset of asthma

Years	*No.*
0 to 3	41
4 to 7	23
8 to 11	7
12 to 15	0
Total	71

for chronic asthma. The age range was from 2 to 15 years at the time the child entered the study (Table I).

Sex distribution is as follows: boys, 46; girls, 25. Table II shows the range of ages at the time of onset.

Complete medical and allergic history and physical examination were recorded by the allergists. Initial laboratory examinations included a hematocrit, serologic test for syphilis, urine analysis for albumin and sugar, and a chest microfluorogram. These laboratory findings were within normal limits. A nasal smear, stained for eosinophils and bacteria, and a timed vital capacity determination were done on all patients, and the filter paper sweat test for cystic fibrosis on the majority. Gamma globulin determinations were carried out on several of the children and one had a low value. No other complicating illnesses were noted, although bronchiectasis was suspected but not proved in one child. The majority of the patients had been actively followed for 2 to 3 years prior to the study; 14 patients had been followed medically for less than 1 year.

Sixty-one subjects (86 per cent) had a history of a collateral allergy, defined as either hay fever or typical atopic eczema. Sixty-one (86 per cent) also had a positive family history of allergy (hay fever, typical atopic eczema, or bronchial asthma); 2 were adopted and family history was unknown; 8 had a negative family history for allergy. Twenty parents had asthma, 9 mothers and 11 fathers; there were no cases of asthma in both parents.

Etiological agents, as revealed in the histories, are summarized in Table III.

Correlations between skin testing and allergic history were high (Table IV).

Medical therapy consisted of perennial hyposensitization to the specific aeroallergens incriminated by the history and confirmed by significantly positive skin tests for pollens, molds, house dust, and/or miscellaneous inhalants. Antidust programs and dander avoidance were carried out in appropriate cases. Diet manipulation and dietary restrictions were used when indicated. Continuous oral bronchodilators in tablet or liquid form were given in appropriate doses after each meal and at bedtime as well as a delayed action oral bronchodilator at bedtime. Since almost all of the children had perennial symptoms, bronchodilators were given on a continuous basis rather than with attacks in the majority of cases. Aerosol bronchodilators and aminophylline suppositories were used on a symptomatic basis. If the infectious component was prominent in triggering asthmatic episodes, the addition of a stock bacterial vaccine was included in the hyposensitization program and an adenovirus vaccine was also employed in certain cases. Appropriate antibiotics were used to treat intercurrent infections. A few patients had also received gamma globulin injections in the past; however, serum gamma globulins were normal in all the patients on whom levels were determined, except for the one case cited.

On each visit to the allergist's office, in

Table III. Etiological agents by history

	No.	*%*
Allergic factors alone	17	25
Infectious factors alone	8	11
Both allergic and infectious	43	60
No clues	3	4
Total	71	100

addition to the usual history and pertinent physical examinations, height and weight measurements and timed vital capacity were recorded. These factors, as well as the total medication required and the amount of school time lost because of asthma, were considered of major relevance in the assessment of the severity of asthma and the response to medical management.

CRITERIA FOR DETERMINATION OF SEVERITY

The severity of asthma was graded according to the following criteria:

Mild, asthmatic attacks infrequent and relatively easily controlled.

Moderate, asthmatic attacks moderately frequent, often requiring epinephrine injections and occasionally hospitalization.

Severe, asthmatic attacks which have led to pulmonary emphysema; and/or minimal response to sympathomimetic amines with repeated hospitalizations; and/or resulting steroid medications.*

Three separate assessments of severity were made in each case, referring to the following intervals or point of time:

I. From onset of asthma to date of first referral to the allergist, usually a period of several years. The estimate is an over-all one covering the full period.

II. From date of first referral to the allergist to date of research psychiatric study, an estimate of then current status.

III. Follow-up 1 to 2 years following research psychiatric study.

RESPONSE TO TREATMENT

Response to medical management was graded according to the following criteria:

Good. Remission or significant reduction in severity or frequency of attacks; absence from school due to asthma rare.

Moderate. Reduction in severity and frequency of attacks; absence from school due to asthma reduced at least 50 per cent.

*Steroids were used as little as possible, and only if all other therapy had failed repeatedly; only 5 children in this series received steroids for prolonged periods.

Table IV. Correlations between skin tests and asthma history

	No.	*%*
Positive tests with definite correlations to asthma history	44	62
Positive tests with equivocal correlations to asthma history	16	22
Positive tests with correlation to hay fever symptoms alone	5	7
No correlation between skin tests and history	6	9
Total	71	100

Minimal. Lesser reduction in severity and frequency of attacks; absence from school due to asthma reduced less than 50 per cent.

Response to medical management was assessed for two periods: (A) from date of first referral to the allergist to date of research psychiatric study (usually several years) and (B) from date of study to date of follow-up (1 to 2 years).

PSYCHIATRIC ASSESSMENT

Our major interest from the psychiatric standpoint was in evaluating relationships between the child's asthmatic condition and his life experience, particularly within the family. This, we believed, could best be done through the systematic study of specific factors thought to be most relevant to the child's psychological situation. The factors chosen related for the most part to quality of relationship experiences within the family, parental attitudes, patterns of child care, and the child's personality and general adjustment.

In each case the medical history was reviewed in detail by the allergist and the psychiatrist jointly. Later 69 mothers and 50 fathers were interviewed in 2-hour sessions by separate members of the team. We were interested in learning what we could about the parents' backgrounds,* their personalities, the tone of relationships in the home, methods of child care, attitudes toward the child and his asthma, and the

*Range of socioeconomic level was wide, with all levels represented.

Table V. Severity of asthma

	I Prestudy	*II Time of study*	*III Follow-up**
Mild	15	29	32
Moderate	28	25	21
Severe	28	17	11
Total	71	71	64

*Seven children were not available for follow-up.

effect of the child's illness on family living. The patient's longitudinal history was reviewed with emphasis on early relationships and experiences and the effects of the illness on adaption. Because many of the children and their families had been known to the allergists for some years, there was already much significant material in the records. The interviews were relatively unstructured and spontaneous, but each interviewer attempted, at appropriate points, to cover specific areas that could later be codified.

As we reviewed the total material on each family it was necessary to attempt to distinguish etiological from reactive influences. In many cases we were impressed with the degree to which the family dynamics and the pattern of family living had been altered because of the presence of the chronically ill child. In our interviews with the parents many felt free to express feelings of frustration, disappointment, sometimes anger, or martyrdom. We did not interpret these expressions *in themselves* as indicators of a negative relationship with the child but evaluated them in the light of the total family tone and relationship qualities.

Each child was seen in psychiatric interview appropriate to his age, by 1 or 2 of the team members. In addition to a personality evaluation we were interested in assessing the child's attitude toward his asthma, the uses he made of the illness, and his feelings about medical management.

Seventy-one variables relating to the family situation, the child's adjustment, and the asthma situation were recorded on check sheets and then the ratings were transferred to IBM cards.

ANALYSIS OF DATA

Statistical analysis involved 3 related procedures:

A. Exploration of relationships between variables.

B. Comparison of 2 subgroups representing extremes of family adjustment, including relationships, stability, and child care.

C. More detailed analysis of specific areas relating to asthma, involving comparison of 3 subgroups differentiated on the basis of family relationships and stability.

A. Relationships between variables.

Procedure. This represented a lead-seeking approach in which relationship of one variable to another in the total series was considered. For example, such factors as warmth of family relationships, parental characteristics, marital relationships, maternal overprotection, rejection, and many others were each compared with such factors as the child's general adjustment, peer adjustment, school adjustment, and severity of asthma and response to medical management. A measure of the significance of relationships was obtained applying chi square to the distribution of the total number of subjects in 2×2 contingency tables.

We were interested in findings involving 3 groups of variables: (1) those relating to the family situation; (2) those reflecting the child's adjustment; and (3) those relating to the asthma situation. Table VII shows the p values of chi squares assessing relationships of 16 variables, 6 of the family situation, 4 of the child's adjustment, and 6 of the asthma situation. Of the 71 variables these are considered most relevant to the two hypotheses.

Results. As will be seen in Table VII, of the 60 chi squares measuring significance of association between the 6 asthma and the 10 family and child adjustment variables, none shows statistical significance. On the other hand, of the 45 chi squares assessing relationships between family situation and child's adjustment variables, 37 show significant p values. These findings then fail to support our first hypothesis, while lending strong support to the second. The ex-

pected relationship between the family situation and the child's asthma situation is not demonstrated; the expected relationship between the family situation and the child's adjustment is demonstrated. These interesting, if disappointing, findings led us to approach the data through a more circumscribed and definitive statistical procedure.

B. Comparison of 2 subgroups.

Procedure. It was expected that greater concordance between the allergic and the psychiatric situations might be demonstrated by comparing the 2 groups representing the extremes of family adjustment. The 71 families were placed on a continuum from positive to negative adjustment based on criteria drawn from the interviews and the histories. Six factors were recorded on a chart, each according to a 5-point scale: (1) adjustment of mother, (2) adjustment of father, (3) marital relationship, (4) mother-child relationship, (5) father-child relationship, and (6) family tone.

At least 2 charts were compiled for each family, one each by separate interviewers, and, where disagreement appeared, consensus was arrived at through discussion; the level of concordance was high. The graphs were used to sort the families and the 20 most positive and the 20 most negative were selected for statistical study. The fact that only the extremes were used gave confidence to the discreteness of the groups. It was much less likely that errors in judgment would lead to misplacement of families where only the extremes were included. The 31 median families were not utilized in this second assessment.

Selection of cases from extremes of a continuum constitutes methodologic control of one variable, in this case the continuum having to do with interpersonal adjustment within families. The distribution of subjects on this variable having been dichotomized, comparison of the groups on another variable permits use of such statistical techniques as the chi square to assess their independence.

Comparison of Group G (good adjustment) with Group P (poor adjustment) was confined to 23 variables, whose distributions were also dichotomized. The distribution of the 40 cases in the resulting 2 × 2 tables was analyzed with the use of the chi square as a test of independence. Table VIII shows the results of this assessment.

Table VI. Response to medical management

	A *Prestudy*	*B* *Follow-up**
Good	21	27
Moderate	25	21
Minimal	25	16
Total	71	64

*Seven children were not available for follow-up.

The table indicates that, again, no significant difference is found between the P and G groups in factors relating to the child's asthma, including age at onset, severity, and response to treatment. In the areas of the child's adjustment there is a highly significant difference between the P group and the G group; the expected correlation between relationships and experience within the family and the child's personality and adjustment is found. The findings, then, again fail to support our first hypothesis while they do support the second.

C. Investigation of changes in asthma from initial to follow-up assessments.

Procedure. Earlier, in Table V, the differences in level of severity in the total sample at 3 time intervals were indicated. Also in Table VI progress in response to medical management for the total sample was indicated. We were now interested in comparing our groups for these changes. For this assessment median families were included, providing 3 groups of families for comparison: 17 with poorest adjustment, 20 with the most positive adjustment, and 27 with median adjustment. The original total of 71 cases was reduced to 64; 7 children were not available for follow-up.

The 3 groups were compared in terms of the direction and pattern of changes. The question whether the patterns of change differ is evaluated with the use of chi square as a test of goodness of fit. In other words, does the distribution of changes for any

Table VII. P values showing significance of relationships assessed from chi squares (N = 71)

	VARIABLES	Relationship of parents	Tone of family relationships	Disturbance in mother	Disturbance in father	Effect of asthma on existing family disturbance	Quality of parent-child relationships	General adjustment	School adjustment	Relationship with peers	Relationship with siblings	Age at onset of asthma	Severity of asthma: initial (1)	Severity of asthma: middle (2)	Severity of asthma: follow-up (3)	Response to treatment: initial (1)	Response to treatment: follow-up (2)
FAMILY	Relationship of parents	.001															
	Tone of family relationships	.001	.001														
	Disturbance in mother	.001	.001	.001													
	Disturbance in father	.001	.005	.001	.001												
	Effect of asthma on existing family disturbance	.001	.001	.001	.001	.005											
	Quality of parent-child relationships	.001	.005	.001	.001	.001	.001										
CHILD	General adjustment	.005	.005	.001	.01	*	.001										
	School adjustment	*	*	.005	*	.025	.05	.001									
	Relationship with peers	.005	.005	.025	.025	.005	.001	.001	.001								
	Relationship with siblings	.001	.01	*	.001	*	.001	.005	*	.001							
ASTHMA	Age at onset of asthma	*	*	*	*	*	*	*	*	*	*						
	Severity of asthma: initial (1)	*	*	*	*	*	*	*	*	*	*	.001					
	Severity of asthma: middle (2)	*	*	*	*	*	*	*	*	*	*	*	.001				
	Severity of asthma: follow-up (3)	*	*	*	*	*	*	*	*	*	*	*	.025	.001			
	Response to treatment: initial (1)	*	*	*	*	*	*	*	*	*	*	*	*	*	.001	.001	
	Response to treatment: follow-up (2)	*	*	*	*	*	*	*	*	*	*	*	*	*	.001	.001	.001
		FAMILY						CHILD				ASTHMA					

*Indicates high p values and lack of significant relationships.

Table VIII. Chi square and p values showing comparison of groups' distributions on selected variables

Variable	Dichotomous categories	Groups P	Groups G	Chi square*	p value*
Family structure					
Age	0 to 7	11	10	—	—
	8 to 15	9	10		
Sex	Male	14	13	—	—
	Female	6	7		
Place in family	Oldest and only	16	8	6.67	.01
	Middle and youngest	4	12		
	Oldest	14	6	4.92	.05
	Only, middle, and youngest	7	13		
No. of children	1 or 2	13	10	—	—
	3 or more	7	10		
Mother's age	20 to 49	20	20	—	—
	50 plus	0	0		
Father's age†		19	20	—	—
		1	0		
Family adjustment					
Tone of family relationships	Warm	2	19	29.32	.001
	Moderate and cold	18	1		
	Warm and moderate	15	20	—	—
	Cold	5	0		
Parents' relationship	Good	0	19	36.20	.001
	Moderate and poor	20	1		
	Good and moderate	5	20	24.00	.001
	Poor	15	0		
Disturbance in mother†	Mild and none	7	19	18.51	.001
	Severe	13	0		
	None	1	15	22.05	.001
	Severe and mild	19	4		
Disturbance in father†	Mild and none	9	20	11.54	.001
	Severe	10	0		
	None	2	14	14.21	.001
	Mild and severe	17	6		
Parents' relationship with child	Not disturbed	1	18	28.98	.001
	Mildly and very disturbed	19	2		
	Not and mildly disturbed	7	20	19.26	.001
	Very disturbed	13	0		
Mother's attitude re asthma	Accepts	12	20	7.66	.01
	Rejects	8	0		
Asthma's effect on existing family disturbance	None	0	13	22.94	.001
	Marked and moderate	15	2		
	None and moderate	11	20	9.18	.005
	Marked	9	0		
Child's adjustment					
General adjustment	Good and average	6	20	21.54	.001
	Poor	14	0		
	Good	2	15	17.30	.001
	Average and poor	18	5		
School adjustment	Good and average	8	19	5.16	.05
	Poor	12	1		
	Good	6	14	6.40	.02
	Average and poor	14	6		

Table VIII. Cont'd

Variable	*Dichotomous categories*	*Groups*		*Chi square**	*p value**
		P	*G*		
Child's adjustment (cont'd)					
Peer adjustment	Good	2	18	25.60	.001
	Average and poor	18	2		
	Good and average	10	20	17.28	.001
	Poor	10	0		
Relationship with siblings‡	Not and mildly disturbed	13	19	3.97	.05
	Very disturbed	5	0		
	Not disturbed	3	13	10.08	.01
	Mildly and very disturbed	15	6		
Asthma					
Age at onset	0 to 7	18	18	—	—
	8 to 15	2	2		
Severity, initial	Moderate and mild	16	11	—	—
	Severe	4	9		
	Mild	4	4	—	—
	Moderate and severe	16	16		
Severity, middle	Moderate and mild	16	15	—	—
	Severe	4	5		
	Mild	7	10	—	—
	Moderate and severe	13	10		
Severity, follow-up§	Moderate and mild	16	15	—	—
	Severe	1	5		
	Mild	8	11	—	—
	Moderate and severe	9	9		
Response to treatment, initial	Good and moderate	10	13	—	—
	Poor	10	7		
	Good	6	6	—	—
	Moderate and poor	14	14		
Response to treatment, follow-up	Good and moderate	12	15	—	—
	Poor	5	5		
	Good	5	8	—	—
	Moderate and poor	12	12		

*Blank spaces indicate that the difference between groups is not statistically significant.
†Information not available on some subjects.
‡No siblings in 3 cases.
§Frequencies less for Group G because 3 subjects had no follow-up.

group show statistically significant deviation from the others? In this procedure the pattern of changes for each group is tested against the combined pattern of all 3 groups using chi square. The total distribution is taken as the best estimate of expected changes against which the changes for each group can be compared.

Results. As can be seen from Tables IX and X differences are slight between the distributions of changes in the 3 groups. Statistical testing of these differences shows no significant deviations. Thus it is concluded that all 3 groups show essentially the same distribution of changes in severity of asthma and response to treatment.

DISCUSSION

Our earlier clinical impressions that led to the hypothesis of a positive relationship between disturbances in family dynamics and the asthma situation in children failed to find support when the data were exposed to statistical analysis. We have been interested to note that the other controlled studies that we have found in the literature

arrived at similar conclusions. Thus Neuhaus[17] reports lack of significant differences in projective test results between children with asthma and heart disease and between the sick children and their well siblings. More recently, Fitzelle[9] found no significant differences in results in personality tests between parents of asthmatic children and parents of a control group, and their attitudes toward child rearing were not significantly different. Fitzelle also compared the parents of a small group of children with severe and mild asthma; as in our sample, no statistically significant differences appeared.

Failure to confirm assumptions that we had tended to take for granted has been disconcerting but should not be too surprising. We have reached a point in child psychiatry where many clinical impressions must be exposed to disciplined evaluation and some negative findings must be expected. The fact that our data demonstrate a close relationship between family dynamics and personality and behavior in children is reassuring; we would indeed be at a loss if this second hypothesis were not upheld. The fact that this second relationship is so positive intensifies and makes more meaningful, we believe, the negative findings relating to the direct role of family dynamics in asthma and tends to lend confidence to the validity of our family evaluations.

Table IX. Frequency of changes in severity of asthma, initial to follow-up

	Groups			
	Poor adjustment	*Moderate adjustment*	*Good adjustment*	*Total*
Improved	8	14	8	30
No change	9	11	12	32
Worse	0	2	0	2
Total	17	27	20	64

Table X. Frequency of changes in response to treatment, initial to follow-up

	Groups			
	Poor adjustment	*Moderate adjustment*	*Good adjustment*	*Total*
Improved	6	9	2	17
No change	10	14	17	41
Worse	1	4	1	6
Total	17	27	20	64

We have looked into our earlier material in an attempt to understand the reasons for our apparently unfounded assumptions. A number of factors appear important:

1. As noted at the beginning, as psychiatrists, we had tended to generalize from a highly selected group of asthmatic children, those with intractable asthma or severely disturbed children with coincidental asthma. We are probably much more prone than we at times realize to overgeneralize from selected samples in our psychiatric work and it is likely that this has led to distortions in emphasis in many clinical areas, especially in relation to the etiology.

2. We have probably been insufficiently sensitive to the effects of a child's chronic asthma on family relationships, viewing reactive patterns as etiological. Reviewing our techniques, we have been struck by the possibility that we have tended to interpret content in regard to asthma somewhat differently, depending on the focus and context of the interview. When a disturbed mother of a disturbed child with asthma describes her attitudes toward the illness, we think traditionally in terms of primary cause and effect. When an intact mother with a well-adjusted child describes similar attitudes we tend to see them more as reactive to hardships that the illness may impose. Both mothers may express irritation with the child's nocturnal attacks. Does the fact that one family is disturbed and the other well adjusted, in itself, justify a different interpretation of the interview content in relation to effects on the child's illness? The principle implied is subtle but of major importance in psychophysiologic research.

3. Related to the previous factor has been our tendency to be too ready to assume a direct link between the dynamic situation

within a family and a clinical syndrome in the child. We have not taken seriously enough Whitehorn's[23] caution, reiterated by Bender,[2] that a dynamic description may be thoroughly accurate but still may not be etiologically related to the specific illness or disturbance presented by the child.

4. In our need for a sense of definitiveness, in a field still uncertain in so many areas, we have tended to incorporate prematurely as "knowledge" formulations insufficiently verified and at times presented by their authors as speculations.

While the main purpose of this paper has been to report on the statistical study, we think that some brief clinical comments are indicated. Although we were unable to demonstrate a specific relationship between family pattern and onset, course, and severity of the asthma in an unselected group of patients, this does not imply a lack of need for insight into the psychological realities in the life of the asthmatic child and his parents. The cases in this series present striking examples of ways in which asthma has affected family living adversely. In many homes major housekeeping and other physical changes were necessitated, long hours of otherwise useless drudgery imposed on mothers, many sleepless nights exacted, numerous and often expensive trips to doctors and hospitals called for. Beyond these physical demands were anxiety, in some families severe and ever present, and a sense of frustration because the trouble seemed to have no ending regardless of what was done. It is evident that these factors of fatigue, irritability, anxiety, and financial strain all play a role in coloring the parent-child relations as they evolve through the course of the disease.

Of more serious import is the high degree of negative effect of the child's illness on already existing family disturbances. It is evident that in some cases, despite personality difficulties in the parents and relationship problems, the families were managing to adjust reasonably well until the extra strain of having to live with chronic asthma was added. Conflicts, previously defended against, came to the surface and major difficulties resulted. This was true in situations such as that of older parents who had previously raised children successfully and then could not adjust to severe asthma in the last child; immature younger parents who did not have the resiliency to deal with the extra burdens that asthma in the child brought to the marriage; parents who themselves had been or were asthmatic and as a reaction had particular difficulty accepting the illness in the child; and parents who were overwhelmed with guilt and a disabling sense of personal responsibility for the child's illness. Because of its nature and attendant problems, asthma tends to place a greater strain on family living than most chronic illnesses and there is a particularly urgent need to control it medically and reduce chronicity wherever possible.

Another important consideration is the marked anxiety experienced by mothers in reaction to attacks, despite repeated episodes. The question of overprotectiveness was somewhat difficult to evaluate because in many cases restrictions had to be imposed on activities. Infection was a major trigger and parents learned the necessity for precautionary measures. Overprotectiveness was much the exception although a small number of overanxious, infantilizing mothers went to almost incredible extremes of restriction.

As to the children themselves, no suggestion of a characteristic personality profile emerged. The range of personality, behavior, and general adjustment was broad. As indicated in the statistical data, and, as expected, the children with the most positive family relationship experiences and care tended to be the most comfortably adjusted with the converse being also true.

Content concerning the meaningfulness of asthma to the children was particularly interesting from a psychiatric viewpoint. Our earlier experience had indicated that patients referred to the psychiatrist because of obvious severe emotional difficulties along with asthma tended to demonstrate a high level of anxiety, fear of suffocation and death, and other morbid preoccupations. In

this unselected group, interestingly, such severe reactions were much less frequent. A major factor in reducing fear was the availability of immediate relief through medication. The reality threat was minimized as the child developed confidence in the medication. Where response to drugs in the home was less certain, anxiety and its disabling elaborations were more common. The children's most commonly expressed feeling about the illness was one of frustration; it interfered with achievement of goals, with the realization of plans, and with social life. It was often more resented than feared. This was not true of the more disturbed children in whom preoccupation with the illness tended to become a vehicle for the expression of their psychopathology.

The question was raised in our material as to the patients' use of asthma for secondary gains, to manipulate situations, to escape responsibility, to gain one-to-one attention, and similar goals. In general these mechanisms were observed to a significant degree only in those children whose basic needs had been unmet or only partially gratified, with the asthma used as part of a total adaptation pattern.

Finally, do these statistical and clinical findings have implications for child psychiatry in its relationship with allergy? It would seem most important that we recognize that the negative statistical findings do not in any way imply that the family situation does not have a direct effect on the adjustment of the child with asthma, on his adaptation to the illness, and on the level of limitation the asthma imposes. These factors must be differentiated from effects on the course of the asthma itself. Failure to make this differentiation has led to much confusion in the literature.

SUMMARY

The first phase of a research project on childhood asthma carried out jointly by allergists at the University of Michigan Medical Center and child psychiatrists at Hawthorn Center is described. The focus of the present investigation is the relationship between family psychological situation and the child's asthma situation. Seventy-one children with chronic bronchial asthma and their families were studied to test the following hypothesis:

A study of asthmatic children and their families will reveal a positive relationship between the asthma situation (onset, severity, response to treatment) and the family situation (quality of family relationships, identification opportunities, and care and handling).

As a control a second hypothesis was tested: *A study of the same group of children and their families will reveal a positive relationship between level of disturbance in the child's personality and/or behavior, and level of disturbance in family relationships, and care and handling.*

The patients were drawn from the regular office and clinic patients of practicing allergists. The asthma situation was studied with emphasis on severity, course, and response to medical management over a period of at least 2 years. Psychiatric study involved individual interviews with each parent and with each patient and systematized recording of findings. Seventy-one variables relating to family situation, the child's adjustment, and asthma situation were recorded for statistical analysis.

Three statistical assessments of the data were made:

1. Exploration of relationships between variables. No significant relationships between variables of the family situation and those of the child's asthma are found. In contrast, there is frequent occurrence of significant correlation of variables of the family situation and those of the child's adjustment. Thus the first hypothesis is not supported, while the second hypothesis is strongly supported.

2. Comparison of 2 subgroups representing extremes of family adjustment, including relationships, stability, and child care. The 20 families showing best adjustment and the 20 families showing poorest adjustment are compared on 23 variables including those dealing with severity of asthma and response to treatment. The independent grouping of

subjects is confirmed by consistently significant contingencies for all variables concerned with family adjustment. The first hypothesis is not supported; no differences are found between the 2 extreme groups on the asthma variables. The second hypothesis is strongly supported; there is positive relationship between family pattern and child personality and adjustment.

3. **Three groups differentiated on the basis of good, moderate, and poor family situation are compared on the changes shown in severity and response to treatment from initial to follow-up status.** None of the 3 groups shows significant differences from each other in pattern of changes.

All these statistical explorations lead to the conclusion that, in the areas of severity of asthma and response to treatment, no relationships with family situation are demonstrable. On the other hand, close relationships between family situation and child's adjustment are consistently demonstrated by the findings.

Child psychiatry is seen to have a selective role in the treatment of the asthmatic child. Routine referral is unnecessary. On the other hand, the disturbed asthmatic child requires psychiatric help, and medical-psychiatric treatment is clearly indicated for a good many of the parents. The deciding factor is not the child's asthma but the emotional status of both child and family, with the recognition that the asthma may add new problems requiring therapeutic intervention through the coordinated efforts of allergists, child psychiatrists, and case workers.

REFERENCES

1. Bakwin, R. M.: Symposium on Pediatric Allergy; Essentials of Psychosomatics in Allergic Children, Pediat. Clin. North America **1:** 921, 1954.
2. Bender, L.: Psychological Problems of Children With Organic Brain Disease, Am. J. Orthopsychiat. **19:** 404, 1949.
3. Bernstein, L., Allen, J. E., Kreindler, L., Ghory, J. E., and Wohl, T. H.: A Community Approach to Juvenile Intractable Asthma, Pediatrics **26:** 586, 1960.
4. Bostock, J.: Asthma: a Synthesis Involving Primitive Speech Organism and Insecurity, J. Ment. Sc. **102:** 559, 1956.
5. Creak, M., and Stephen, J.: The Psychological Aspects of Asthma in Children, Pediat. Clin. North America **5:** 738, 1958.
6. Dunbar, F.: Emotions and Bodily Changes, New York, 1954, Columbia University Press.
7. Dunbar, F.: Psychosomatic Diagnosis, New York, 1943, Paul B. Hoeber, Inc.
8. Dunbar, F.: Synopsis of Psychosomatic Diagnosis and Treatment, St. Louis, 1948, The C. V. Mosby Company.
9. Fitzelle, G. T.: Personality Factors and Certain Attitudes Toward Child Rearing Among Parents of Asthmatic Children, Psychosom. Med. **21:** 209, 1959.
10. French, T. M.: Psychogenic Factors in Asthma, Am. J. Pyschiat. **96:** 87, 1939.
11. French, T. M., and Alexander, F.: Psychogenic Factors in Bronchial Asthma, Psychosom. Med., Monographic Series, vols. I and II. Washington, D. C., 1941, National Research Council.
12. Leigh, D.: Asthma and the Psychiatrist: a Critical Review, Internat. Arch. Allergy **4:** 227, 1953.
13. McNemar, Q.: Psychological Statistics, New York, 1949, John Wiley & Sons, Inc.
14. Miller, H., and Baruch, D. W.: The Emotional Problems of Childhood and Their Relation to Asthma, A. M. A. J. Dis. Child. **93:** 242, 1957.
15. Miller, H., and Baruch, D. W.: Psychosomatic Studies of Children With Allergic Manifestations, Psychosom. Med. **10:** 275, 1948.
16. Miller, H., and Baruch, D. W.: A Study of Hostility in Allergic Children, Am. J. Orthopsychiat. **20:** 506, 1950.
17. Neuhaus, E. C.: A Personality Study of Asthmatic and Cardiac Children, Psychosom. Med. **20:** 181, 1958.
18. Peshkin, M. M.: Intractable Asthma of Childhood, Internat. Arch. Allergy **15:** 91, 1959.
19. Peshkin, M. M.: Role of Environment in the Treatment of a Selected Group of Cases, Am. J. Dis. Child. **39:** 774, 1930.
20. Peshkin, M. M., and Tuft, H. S.: Rehabilitation of the Intractable Asthmatic Child by the Institutional Approach, Quart. Rev. Pediat. **2:** 7, 1956.
21. Rogerson, C. H.: The Psychological Factors in Asthma-Prurigo, Quart. J. Med. **30:** 367, 1937.
22. Rogerson, C. H., Hardcastle, D. H., and Duguid, K.: A Psychological Approach to the Problem of Asthma and Asthma-Eczema-Prurigo Syndrome, Guy's Hosp. Rep. **85:** 289, 1935.
23. Whitehorn, J. C.: The Concept of "Meaning" and "Cause" in Psychodynamics, Am. J. Psychiat. **104:** 289, 1947.

MEDICAL PROGRESS

Chromosomal aberrations in man

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IN RECENT years, concepts and techniques in genetics have had an increasing influence upon medical thinking, particularly in pediatrics, since genetically determined diseases most often appear early in life and pursue a chronic course. Within the last several years, it has become apparent that chromosomal aberrations are an important new category of genetic disease. An understanding of these problems involves concepts which differ somewhat from those usually incorporated into medical genetic teaching. The purpose of this paper is to review developments which led to the recognition in humans of syndromes associated with identifiable chromosomal changes and to provide some background for an appreciation of future developments in this rapidly changing field of investigation.

Historically, two independent lines of research provoked the recent burgeoning of interest in this field. First were the studies of sex chromatin initiated by Barr and Bertram[1] in 1949. Second was the renewed interest in human chromosomes stimulated by Tjio and Levan[2] who, in 1956, reported methods permitting the identification of individual chromosomes of the human set and establishing the number as 46 instead of 48.

While studying neuronal cells in cat brains, Barr and Bertram[1] found in the female, but not in the male, that a high proportion of cells contained a peripheral nuclear chromatin mass, termed sex chromatin. This correlation between a nuclear structure and phenotypic sex was subsequently observed to be valid for many tissues from many species, including man.[3] Starting in 1954, nuclear sexing was applied to humans with sexual anomalies. It was soon discovered that at least 3 well-recognized syndromes exhibited an apparent sex reversal.[4-10] Both Turner's syndrome and the testicular feminization syndrome were found to be associated with a "male" pattern of nuclear chromatin, whereas Klinefelter's syndrome was associated with a "female" pattern.

Detailed studies of chromosomes in humans have since demonstrated that only limited

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predictions concerning the sex chromosome complement are possible from a consideration of sex chromatin; hence, the terms chromatin positive and chromatin negative are preferable to female and male nuclear pattern respectively. Similarly, the term sex reversal may be quite misleading.

After discussing the problems of chromosome number and morphology, we will review the recent literature dealing with reports of chromosomal abnormalities in humans.

I. THE CHROMOSOMES OF MAN

A. Chromosome number. The chromosome number of man is now firmly established as 46, the haploid complement as seen in germ cells consisting of 22 autosomes, plus an X in the female and an X or a Y in the male.[2, 11-13]

It is interesting that after nearly 30 years when 48 chromosomes were considered the norm, the problem should have been reopened. This was due to a combination of new and older, previously neglected techniques,[14] which included recent advances in tissue culture methods, prefixation treatment with hypotonic saline, use of colchicine to obtain a larger number of metaphase figures, and the use of squash or smear preparations in preference to sectioned material.

Both short-term and long-term tissue cultures have been utilized as well as direct investigation of smear preparations. Tissues studied have been either skin, white cells from peripheral blood, or bone marrow. The most promising techniques for general clinical use are those utilizing peripheral blood.[15]

B. Chromosomal morphology in man. Chromosome numbers per se are inadequate for purposes of characterizing a species. More specifically, the karyotype, embodying information concerning specific morphologic details of individual chromosomes, defines the complement. For this purpose, the chromosomes of somatic cells are studied at metaphase or anaphase. During these mitotic stages, the chromosomes are maximally contracted, attaining a length that is quite constant from cell to cell of individual tissues but may vary in different preparations depending upon the state of condensation and the nature of the prefixation treatment.

At a meeting in Denver in 1960, a study group proposed a standard system of nomenclature of the human mitotic chromosomes.[16] The identification of individual chromosomes is based upon their length, relative to the total haploid set, and the position of the kinetochore or centromere. (The term kinetochore is preferred to the older, more widely used term, centromere, since the latter implies a central position which is often not the case, while the former conveys the correct idea of a role in the movement of chromosomes.)

The kinetochore itself is not readily visualized, but its presence determines the location of the primary constriction which divides the chromosomes into 2 arms. This constriction may vary in position from the center almost to the tip but remains constant for each individual chromosome. The kinetochore controls the movement of chromosomes during cell division and is, therefore, an essential feature. When the kinetochore is centrally located, the arms are of equal length and the chromosome is described as metacentric. A submedian chromosome has arms of unequal length, the larger being some 2 to 4 times as long as the shorter. The kinetochore of an acrocentric chromosome is located almost terminally. It is generally agreed that a second arm is always present; kinetochores are never precisely terminal.

Based upon these parameters, a tentative identification scheme has been established. The autosomes have been serially numbered 1 to 22, as nearly as possible in descending order of length, and the sex chromosomes are referred to as X and Y rather than by a number. The autosomes have been classified into 7 readily distinguishable groups. Within the group containing chromosomes 6 to 12 and including the X-chromosome, it is difficult to single out individual members. However, it is believed that, with favorable

Table I. Human chromosome groups

Group 1-3	Large chromosomes with approximately median kinetochores
Group 4-5	Large chromosomes with submedian kinetochores
Group 6-12	Medium-sized chromosomes with submedian kinetochores; the X resembles the longer chromosomes in this group, especially chromosome No. 6; it is difficult to distinguish these two
Group 13-15	Medium-sized acrocentric chromosomes; chromosomes Nos. 13 and 14 have satellites on the short arm
Group 16-18	Rather short chromosomes with approximately median (No. 16) or submedian kinetochores
Group 19-20	Mediocentric short chromosomes
Group 21-22	Very short acrocentric chromosomes; chromosome No. 21 bears a satellite on its short arm; the Y is grouped with these chromosomes

preparations, distinctions can be made between most, if not all, individual chromosomes. However, a certain amount of skepticism may be in order, as pointed out by Patau.[17] He examines, from a methodologic point of view, the use of length measurements for purposes of identification. He states that the claim that every pair of chromosomes can be identified cannot be sustained. The difficulties following from this uncertainty pertain to the identity of the chromosome lost or gained in certain instances, not to the actual change in number. Accurate counts can be made with present methods.

Fig. 1 illustrates the human chromosome set arranged according to the criteria described above. Table I characterizes the 7 groups of mitotic chromosomes.

In each plant or animal species, one or more pairs of chromosomes may be associated with the formation of nucleoli, which are nuclear structures rich in ribose nucleic acid, probably important in protein synthesis. Nucleolus formation is always associated with a specific region of the chromosome pair or pairs involved. The nucleolar-organizing zone is constricted during metaphase and stains poorly. Chromosomal material distal to this region is sometimes known as a satellite. Ferguson-Smith and Handmaker[18] conclude that at least 5 pairs of autosomes—13, 14, 15, 21, and 22—are satellited. Furthermore, they describe a characteristic association of satellited chromosomes in connection with nucleolus formation which is proposed as a cause of chromosomal anomalies.

Heterochromatic regions have not figured in the discussion of chromosome characteristics thus far, since they are ordinarily visualized only in prophase stages, and standard nomenclature is based on metaphase and anaphase. However, heterochromatin is of great significance in understanding the nature of sex chromatin. During interphase or prophase, heterochromatin can be recognized because it may be out of phase with the metabolic cycle of other portions of the chromosomes, the euchromatic regions. It can be distinguished cytologically from euchromatin because, when out of phase, it is either more heavily condensed and deeply staining or less condensed and more lightly stained than euchromatin. The former condition is spoken of as positive heteropyknosis, the latter as negative heteropyknosis. Some heterochromatic regions have the property of reversibility, being positively heteropyknotic during one stage of the cell life cycle and negatively so during another stage.[19] Other regions remain positive throughout the cycle.

Heitz[20] based his original description of heterochromatin upon these striking staining characteristics. White[21] has defined it as any chromosomal region which becomes heteropyknotic at some stage in its cycle. Sex chromosomes are frequently almost entirely heterochromatic as is the Y of *Drosophila melanogaster*,[22] *Mus musculus*,[23] *Rattus norvegicus*,[24] and possibly of man.[25] Euchromatin contains Mendelizing genes associated with specific morphologic or biochemical traits. The greater part of heterochromatin, however, seems to have no distinctly discernible genes as judged by classical Mendelian concepts, although the few

mutants known to be located in major heterochromatic regions in Drosophila[26] are, through their phenotypic manifestations, indistinguishable from those in the euchromatic areas. The genetic properties of heterochromatin have been most extensively studied in *D. melanogaster* where its most striking property is the induction and modification of variegation associated with position effect, as will be discussed in a later section. This type of genetic effect has recently been reported in mice.[27]

The terms heteropyknosis and heterochromatin are often confused. Heterochromatin can exhibit the property of heteropyknosis, but whether it does or not follows from the conditions of the individual cell concerned. Thus, in somatic cells, one of the 2 X-chromosomes of the female is positively heteropyknotic, forming the sex chromatin body of interphase and prophase.[28] During these stages of meiosis in female germ cells, however, neither X-chromosome is heteropyknotic. Thus, although the heterochromatic nature of the X-chromosomes has not changed, their manifestation of the property of heteropyknosis depends upon the characteristics of the individual cell.[29] Heteropyknosis is not a constant property of heterochromatin. In the male, the situation is reversed.[29, 30] The X-chromosome of somatic cells in interphase or prophase is not heteropyknotic, whereas the X-chromosome during meiotic prophase forms a positively heteropyknotic body in association with the Y-chromosome (Fig. 2).

C. Sex chromatin. For clinical studies in humans, the nuclear sex chromatin mass is most easily demonstrated in scrapings from the buccal mucosa or in skin taken by biopsy. It is seen as a dark-staining body, often appearing bipartite, about 1 μ in diameter, on or near the nuclear membrane. A presumably related phenomenon discernible in from 2 to 8 per cent of neutrophils in the circulating blood of females is a small protrusion from one of the nuclear lobes known as a drumstick.[31-33]

Until recently, the presence of the sex chromatin mass in cells of females was presumed due to somatic pairing of the X-chromosomes. In the male with only one X-chromosome, it was not expected to be observed. However, in the nuclei of regenerating livers of female laboratory rats, Ohno, Kaplan, and Kinosita[28] observed that in diploid cells only 1 of the 2 X-chromosomes exhibited positive heteropyknosis during prophase. Two of the 4 X-chromosomes in female tetraploid cells behaved in this fashion, whereas none of the X-chromosomes in male diploid or tetraploid cells differed in staining properties from the autosomes. In the presence of 2 sets of autosomes, a single X-chromosome remains isopyknotic to the autosomes. Thus, in normal diploid cells of females, the sex chromatin is a manifestation of a single X-chromosome. In male somatic cells, there is no heteropyknotic X. This observation suggests a difference in the biologic properties of the 2 X-chromosomes of female somatic cells. In female cells during early prophase, the heteropyknotic X-chromosome is folded back upon itself, accounting for the bipartite appearance often seen in the sex chromatin mass. The single-X nature of sex chromatin has since been observed in several other species, including man.[34-37]

D. Chromosomal aberrations. The previous discussion may leave the impression that karyotypes of individual species are unvarying and provide constant criteria for purposes of identification. The constancy of the karyotype implies that the number of genes and their lineal relationship are preserved from one cell generation to the next and from parent to offspring. However, cytogenic studies have indicated that variations in the order of the genes and/or in their number may occur spontaneously or under the influence of ionizing radiations and certain chemicals. These changes may, in turn, be transmitted to subsequent generations.

In general, changes in karyotype involve a gain or loss of genic material or a rearrangement of the order of the genes without loss or duplication. The various types of changes observed to occur spontaneously or

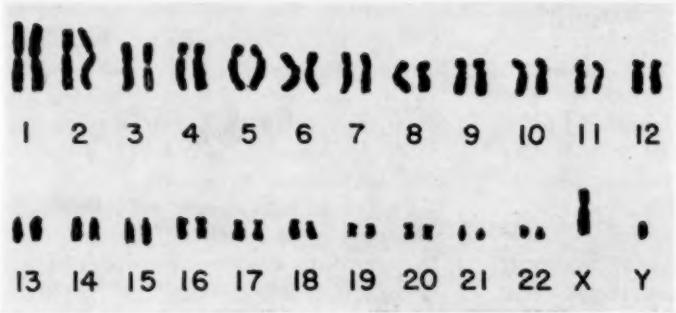

Fig. 1. The human karyotype numbered according to the Denver system. Photograph by Ernest H. Y. Chu.

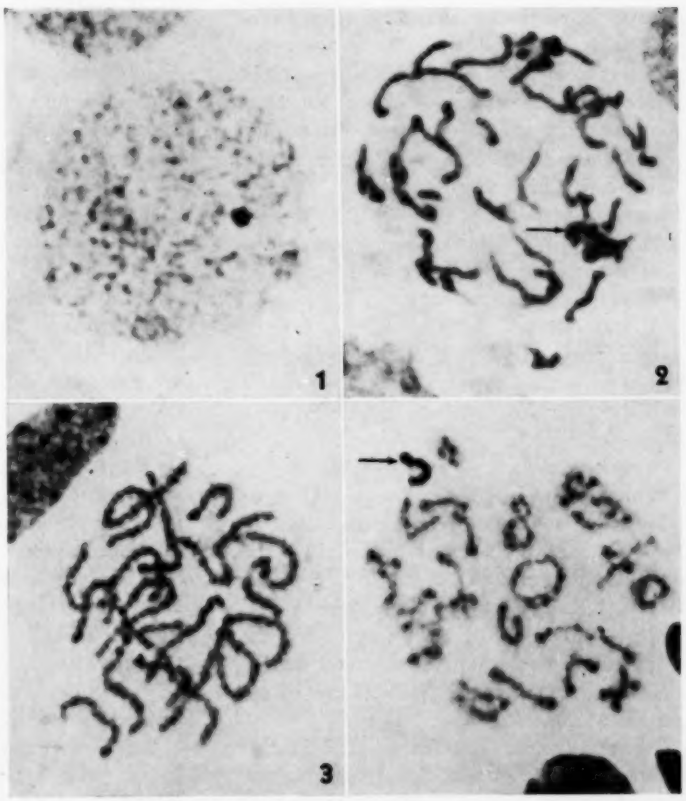

Fig. 2. Sex chromosomes and heteropyknosis. *1*, Sex chromatin in liver cell of human female fetus. *2*, Positively heteropyknotic X-chromosome in early prophase of human fetal liver cell. *3*, Pachytene of female rat meiosis, sex bivalent isopyknotic to autosomal bivalents. *4*, Diplotene of male rat meiosis, heteropyknotic XY-bivalent.

experimentally in a wide variety of plants and animals are outlined as follows.

1. Changes in the number of chromosomes.

A. LOSS OR GAIN OF AN ENTIRE SET. One set in somatic cells, termed haploidy, is unknown in any of the higher animals. One case of triploidy, 3 sets, has been described in cells from skin culture of one individual.[38] Marrow cells, however, were diploid.[39]

B. ANEUPLOIDY. LOSS OR GAIN OF INDIVIDUAL CHROMOSOMES IS CALLED ANEUPLOIDY. Most of the known human anomalies fall into this category. The monosomic condition results from the loss of a single chromosome. The individual is then said to be monosomic for the particular chromosome involved. The only clear example in man is Turner's syndrome, which is associated with the presence of a single X-chromosome. The trisomic condition represents the gain of a single chromosome; these have been described for autosomes and the sex chromosomes. Tetrasomics reflect the gain of 2 of the same chromosome; these have been recognized for the X-chromosome only.

2. Changes in the number or arrangement of genic loci, involving only parts of individual chromosomes.

A. NUMBER.

Deficiency. The loss of one or more genic loci.

Duplication. An increase in one or more genic loci.

B. ARRANGEMENT.

Translocation. The exchange of parts between 2 nonhomologous chromosomes to form a new order of genic material. If the original chromosomes are A B C D E F and G H I J K L, the new ones may be A B C D K L and G H I J E F. Several possible cases now exist in humans and will be discussed in later sections, particularly in regard to Mongolism.

Isochromosome. The fusion of 2 homologous arms of an individual chromosome through misdivision of the kinetochore results in the formation of an isochromosome. By this process, a chromosome with 2 identical arms results. One such instance has been noted in humans.[40]

Inversion. The appearance of 2 breaks within a single chromosome may be followed by the rotation of the broken piece through 180 degrees. By this process, an original gene order of A B C D E F may be changed to produce a new order of A E D C B F. None has been described in humans.

Position effect. Finally, though not yet demonstrated in man, the phenomenon of position effect deserves mention. This is a phenotypic change produced by the transposition of a piece of chromatin material from its usual site within a particular chromosome to a different one within the same or another chromosome of the complement. This has been thoroughly studied in *D. melanogaster* and in the primrose, *Oenothera blandina*.[41]

Two distinct types of position effects are possible, depending on whether the genes transposed and the regions to which they are transposed involve euchromatin or heterochromatin. Position effect studies indicate that the gene does not act independently of other genes but may be actively influenced by the region of the chromosome in which it is located.

E. Mechanisms of aberration.

1. Breakage. This is the basic event which precedes the production of chromosomal rearrangements, deletions, and duplications. In order for a piece of a chromosome to be lost or for a translocation or inversion to occur, breaks must be produced, followed by reattachment and healing. The gain or loss of a whole chromosome, however, involves a different process.

2. *Nondisjunction.* Nondisjunction during germ cell maturation is regarded as the cause of trisomic and monosomic conditions. Our knowledge of nondisjunction is based on genetic studies of *D. melanogaster,* augmented by descriptions of a similar process in a variety of animal and plant species, during meiosis or in somatic cells. The original description in Drosophila was concerned with the paired X-chromosomes of the oöcyte during meiosis.[42] Here it is found that with a frequency of 1 in 2,500 oöcytes, the synapsed X-chromosomes fail to segregate

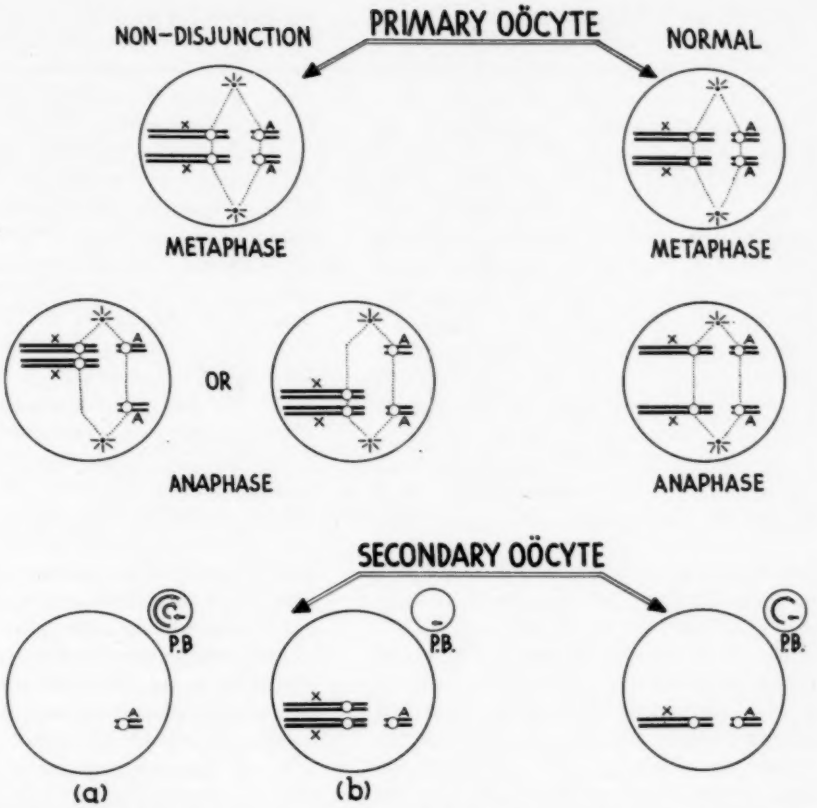

Fig. 3. Nondisjunction in the first meiotic division of an öocyte. In *a* the 2 X-chromosomes have moved into the polar body. The resultant secondary öocyte has no X-chromosome and will give rise to a nullo-X ovum. In *b* the 2 X-chromosomes have moved into the secondary öocyte. The ovum developing from this case will have two X-chromosomes. In both instances the autosomal pair, *A*, has behaved normally.

but move together to the same pole during anaphase. As a consequence, 2 kinds of ova may be produced: those carrying 2 X-chromosomes instead of one or those that carry no X-chromosome. Fertilization by an X- or a Y-sperm of these types of ova results in 4 kinds of abnormal zygotes; XXX, XXY, XO, or YO. XXX flies, trisomic for the X-chromosome but diploid for the autosomes, are poorly viable, although they were called superfemales by Bridges.[43] XXY individuals are fertile females in no way distinguishable phenotypically from XX females. XO flies are monosomic for the X-chromosome and are phenotypically normal males, although sterile since their sperm remain nonmotile. The Y-chromosome by itself in YO individuals is a lethal condition.

Nondisjunction is known to occur also during male meiosis. Following such an event, 2 types of sperm are expected: O-sperm, carrying neither the X- nor the Y-chromosome, and XY-sperm. These 2 types, fertilizing X-ova, could produce XXY individuals or XO individuals. These zygotes would be expected if nondisjunction occurred during the first meiotic division. If taking place during the second division, XX-sperm, YY-

Table II. Expected zygotes

Egg	*Sperm*	*Zygote*	*Phenotype*
a X	a X	XX	Normal female
a X	a Y	XY	Normal male
b No X	a X	X	Turner's syndrome
b No X	a Y	Y	Presumed lethal
b XX	a X	XXX	Triplo-X
b XX	a Y	XXY	Klinefelter's syndrome (chromatin positive)
a X	c XY	XXY	Klinefelter's syndrome (chromatin positive)
a X	c Neither X Nor Y	X	Turner's syndrome
a X	d XX	XXX	Triplo-X
a X	d YY	XYY	Unknown
a X	d Neither X Nor Y	X	Turner's syndrome

a, Products of normal meiosis in male and female
b, Products of nondisjunction in female; meiosis I or II
c, Products of nondisjunction in male; meiosis I
d, Products of nondisjunction in male; meiosis II

sperm, or O-sperm would be expected. These, in turn, fertilizing X-ova, would produce XXX, XYY, or XO individuals.

Fig. 3 represents diagrammatically the process of nondisjunction during meiosis in the human female. Table II illustrates the zygotes expected following nondisjunction in either the female or male. Although the same genotypes are expected as those described for the flies, the resultant phenotypes are markedly different in man, indicating a different balance between male- and female-determining factors in the X and Y than exists in Drosophila.

It seems reasonable to assume that nondisjunction is the cytologic basis for the existence of the XO, XXY, and XXX humans who have been described. We have no definite information concerning the relative frequency of nondisjunction in the male and female of man.

This same reasoning applies to the autosomes. The appearance of autosomal trisomic states may be explained through the mechanism of nondisjunction. Theoretically, either the egg or sperm may be involved. Two types of zygotes could result, one trisomic for a particular autosome, the other monosomic. Since, in man, no monosomic conditions involving the autosomes have been described, and the condition is generally lethal in other species, it is reasonable to suppose that monosomic states will not be found.

The environmental or genetic factors which favor the occurrence of nondisjunction are unknown. In Drosophila, it has been found that increased carbon dioxide and aging of the maternal parent increase the rate of nondisjunction.[44] This association with advanced maternal age is important since Mongolism and possibly Klinefelter's syndrome are known to occur more often in the offspring of older mothers.

3. Mosaicism. This refers to the situation in an individual who possesses at least 2 nonneoplastic, cytogenetically distinct races of cells capable of perpetuating their respective karyotypes. This phenomenon is best explained as arising from a process similar to nondisjunction during mitosis in the developing zygote.

Strictly speaking, nondisjunction refers to the failure of homologous chromosomes to separate during the first meiotic anaphase. Homologues, however, do not come together and synapse during mitosis but act independently of each other. Failure of sister chromatids to separate during anaphase and their consequent movement together to the same pole results in 2 unequal daughter

cells; one trisomic and the other monosomic for the specific chromosome involved.

If this occurs during the first cleavage division, the individual will possess 2 abnormal karyotypes, a probable example of which has been described by Jacobs and co-workers.[45] This would be true unless there is differential viability and the zygote should develop from only one blastomere. Nondisjunction occurring after the first cleavage division could yield an individual with 3 distinct races of cells. If one of the abnormal karyotypes were eliminated, then only 2 races of cells would result, one with a normal and one with an abnormal karyotype. This latter situation is illustrated by the case of Hirschhorn, Decker, and Cooper.[46]

Mosaicism is not always simple to demonstrate, and its interpretation may be uncertain. Court Brown, Jacobs, and Doll[47] have suggested 3 criteria: (1) the number of cells with counts different from the modal number must be more than can reasonably be attributed to chance; (2) this must be demonstrable in more than one examination and preferably in more than one tissue; (3) and finally, cells with an abnormal karyotype should all have the same karyotype.

II. CLINICAL SYNDROMES ASSOCIATED WITH CHROMOSOMAL ABERRATIONS

A. Sex chromosomes.

1. Klinefelter's syndrome. As originally described in 1942, this syndrome was seen in postpuberal males without spermatogenesis but with no absence of Leydig cells, and increased excretion of follicle-stimulating hormone. Their testes were small but of normal consistency, and they had variable degrees of gynecomastia and eunuchoidism. Testicular biopsies revealed hyalinization of the seminiferous tubules and prominent accumulations of Leydig cells.[48, 49]

Interest in this problem was greatly stimulated by reports that some of these patients were chromatin positive.[4-6, 10] Clinical distinctions between the chromatin-positive and chromatin-negative groups are minor, some patients appearing quite normal.[50] However, testicular biopsies can be correlated by experienced observers on the basis of a more characteristic histologic appearance of the testes in the chromatin-positive group.[51, 52] We will confine our remarks to the chromatin-positive group, since the chromatin-negative group possess a normal complement of 46 chromosomes.[47, 53]

Several workers have noted that this syndrome frequently occurs in men of limited intelligence.[52, 54] Prepuberal cases have been most easily detected by surveying sex chromatin patterns in mentally defective male children. Ferguson-Smith[55] examined 668 mentally handicapped school age boys and found 8 (1.2 per cent) whose cells were chromatin positive. All were examined and found to have small testes which, on biopsy, revealed a marked reduction in the number of tubules exhibiting spermatogonia. These children, it is anticipated, will develop Klinefelter's syndrome as they enter puberty.

The most direct estimate of the frequency of the chromatin-positive group was made by Moore[56] who studied oral mucosal scrapings in consecutive newborn infants over a 12-month period at a general hospital. There were 5 instances out of 1,911 births of normal-appearing male infants whose cells were chromatin positive. No chromatin-negative females were detected. Presumably, these chromatin-positive males will develop Klinefelter's syndrome. This suggests a surprisingly high incidence of approximately 1 in 800 births. Combined data on retarded individuals and infertile males lend support to this estimated frequency.[57] By the use of the above figures, it can be further estimated that approximately 7 per cent of persons with Klinefelter's syndrome will be high-grade mentally defective individuals.

Jacobs and Strong[58] first reported in detail an abnormal chromosome number in a chromatin-positive individual with Klinefelter's syndrome. Bone marrow cultures revealed 47 chromosomes, 22 pairs of autosomes plus 2 X's and a Y. The parents of this patient each exhibited a normal complement of 46 chromosomes. This finding has been confirmed by Ford and co-workers[59] and by

others.[53] It seems reasonable to generalize that all chromatin-positive males possess 2 X-chromosomes and one Y-chromosome, and the testicular histologic appearance characteristic of Klinefelter's syndrome.

Presumably, the XXY condition arises as a result of nondisjunction in the germ cells; thus, data on maternal and paternal ages in these conditions are quite important. Ferguson-Smith[60] mentions 22 cases in which the mean maternal age was 33 years. Lenz and his associates[61] report similar findings. Mongolism, another trisomic state, is also associated with advanced maternal age.[62]

Some exceptions to the generalization concerning the association of Klinefelter's syndrome with the XXY condition should be noted. Several cases have been described in which an XX/XXY mosaicism was found in bone marrow.[53] Two individuals have been discovered possessing 3 X's and a Y in addition to a normal autosomal complement.[63] These individuals, who suffered severe mental retardation, had testicular lesions similar to those associated with chromatin-positive Klinefelter's syndrome. In addition, a high frequency of cells with 2 chromatin bodies was noted in mucosal smears, a finding similar to that to be discussed in connection with the triplo-X condition.

Finally, a "double male," a 15-year-old mentally retarded boy with typical chromatin-positive Klinefelter's syndrome, has been described.[64] However, he possessed 48 chromosomes with a sex chromosome constitution of XXYY.

The family history of an individual with chromatin-positive Klinefelter's syndrome is usually negative. Since normal members may be only mildly stigmatized, there is no way to be certain of the validity of the family history unless unmarried and infertile male relatives are examined. In the major published genetic study to date, no affected siblings were found.[65] An excess of childless uncles, paternal in the chromatin-positive cases, was suggested, but the series is too small to be conclusive. The only clearly familial chromatin-positive cases reported have been in identical twins. Those usually cited as indicating a familial tendency are either chromatin negative, without recorded sex chromatin determination or are quite atypical (for example, an association with certain muscular disorders has been suggested[51, 66-68]).

2. *Turner's syndrome.* Turner is generally credited with having established this syndrome as a clinical entity, although his descriptions of 1938 were not the first.[69] The names of Bonnevie and Ulrich are also frequently associated with this condition, which has been plagued with problems of terminology. Pertinent cases are described under a variety of other terms, e.g., gonadal aplasia and stunted growth, ovarian agenesis, primary ovarian insufficiency with decreased stature, syndrome of rudimentary ovaries, and gonadal dysgenesis. In the interest of simplicity, we have retained the eponym of Turner's syndrome.

Since this syndrome consists of a constellation of clinical and pathologic features, many atypical cases have been described. Although chromosome analyses now permit a more exact definition of this syndrome, additional problems of classification arise. In recent years, the term Turner's syndrome has usually been employed to describe phenotypic females who exhibit short stature, absent gonads in association with an immature but normal female genital tract, webbing of the neck, and other less constant congenital anomalies.[51, 70]

That some individuals with Turner's syndrome might be chromosomal males was suggested by Jost's[71] series of experiments which showed that male rabbit fetuses, castrated at a sufficiently early stage, always developed into phenotypic females. With the development of the sex chromatin test, it was thought possible to examine this hypothesis, and in 1954-1955, several investigators described individuals with Turner's syndrome who were chromatin negative.[7-9] Grumbach and Barr[51] have stated that 80 per cent of their series of 250 patients were chromatin negative. Until recently, such individuals were presumed to

be genetic males; hence this syndrome was termed a sex reversal.

In 1959, Ford[72] reported the first instance of chromosome analysis in Turner's syndrome. Their chromatin-negative, 14-year-old girl yielded, in short-term bone marrow cultures, cells with only 45 chromosomes. Detailed studies led to the interpretation that only one sex chromosome, the X, was present. This has been confirmed by other workers.[73, 74] The problem of atypical cases will be considered later, but it is reasonable to conclude that the typical case of chromatin-negative Turner's syndrome will be found associated with the XO state.

Recent descriptions of 3 cases indicate that the XO state may not always result in gonadal agenesis. One 33-year-old, chromatin-negative woman with the clinical features of Turner's syndrome was described who had secondary sex characteristics indicative of ovarian function.[75] There was some pubic hair and breast development. Menstruation commenced at age 14 and persisted regularly until age 20. Her cells contained 45 chromosomes; only a single X was present. The second case was that of a 39-year-old woman, short of stature, with no other stigmas of Turner's syndrome.[76] Secondary sex characteristics were normal, and, at age 31, she gave birth to a normal son. Chromosome studies of the mother revealed an XO complement.

Clinically defined cases of Turner's syndrome in males have shown a normal male complement,[77] except for the report of Bloise and colleagues.[78] The boy described had a rudimentary penis without urethra and separate perineal urethral and vaginal openings. Internally, there was a uterus and a rudimentary testis near one Fallopian tube, and on the other side, a streak of connective tissue in place of an ovary. Chromosome counts on bone marrow cells revealed 44 autosomes and a single X. Studies of skin cells were not done, so the possibility of mosaicism is not ruled out. The detailed clinical description suggests intersexuality, since the only clearly male feature in this case is the testislike gonad.

It is impossible to discuss the significance of chromatin-positive cases of Turner's syndrome without chromosome analyses. The wide range of phenotypic expression of the XO condition and the fact that similar clinical pictures may result from a variety of causes make it imperative to define the karyotype associated with each case. Grumbach, Morishima, and Chu[79] have reported upon a patient with chromatin-positive Turner's syndrome in whom the original chromosome counts in skin cells disclosed a 45-chromosome, XO karyotype. Subsequent chromosome studies of blood cells have revealed, however, that mosaicism is involved.[80]

Fraccaro and co-authors[81] describe 3 cases conforming clinically to Turner's syndrome, with 46 chromosomes where only a single X was identified but with one other unpaired chromosome tentatively described as an isochromosome for the long arm of the X. Essentially, this is an unbalanced condition; trisomy for the long arm and monosomy for the short arm of the X-chromosome.

The spectrum of clinical and cytogenetic findings covered by the term Turner's syndrome, or gonadal dysgenesis, has not been clearly delineated. Not all clinically defined cases of this syndrome are XO. The most constant feature associated with the XO state appears to be short stature. Usually the gonad is absent, but a functional ovary is sometimes present and possibly even a rudimentary testis. More cases are necessary before any generalizations can be made. Moreover, gonadal agenesis or dysgenesis may occur with other sex chromosomal arrangements, including a normal karyotype.

Familial cases have been described, although none were noted in 2 recent reviews comprising 117 cases.[51, 70] Chromosomal analyses may indicate some unique arrangement in familial instances. The maternal age of mothers of chromatin-negative individuals with Turner's syndrome appears to fall within normal limits.[60]

3. Gonadal dysgenesis in phenotypic females. In addition to Turner's syndrome,

there are many clinical entities, even more difficult to classify, characterized by faulty development of the gonad in apparent females. An insight into some of these problems has been provided by chromosomal analysis.

A. Harnden and Stewart[82] have described a case of "pure gonadal dysgenesis" in a tall, eunuchoid, 19-year-old female with primary amenorrhea and infantile secondary sexual characteristics. She was chromatin negative, and was found to have 46 chromosomes with a normal assortment of autosomes and an XY pair. The gonads were not examined. However, similar cases have been reported, some chromatin negative, others positive, in which the gonads were absent or immature.[83-86] These conditions probably result from early gonadal failure, which can occur for many reasons. A phenotypic female with an XY sex chromosome set is perhaps more properly classified as having a sex reversal than Turner's syndrome. As pointed out by Harnden and Stewart, their case is a better analogy to the experiments of Jost, inasmuch as Jost dealt with castrated rabbit fetuses and not chromosomal anomalies.

B. One case of congenital gonadal failure related to a chromosomal aberration has been described by Jacobs and associates.[45] This was a 37-year-old female with a syndrome which seems to lie between Turner's and "pure gonadal dysgenesis." This individual, of low normal intelligence, had primary amenorrhea and no secondary sexual development; the gonads were streaks of rudimentary tissues. Buccal smears revealed that only 7 per cent of the cells were chromatin positive, and the chromatin body itself seemed unusually small. No drumsticks were seen in peripheral blood cells. Forty-six chromosomes were counted; 45 were readily identified and included a normal autosomal complement plus one X-chromosome. The odd chromosome resembled chromosome 16, but was interpreted as being an abnormally small X. Tentatively, this case may be regarded as an illustration of primary gonadal failure in a genetic female due to loss of chromatin from one X-chromosome.

C. The syndrome of testicular feminization has been known to be associated with a chromatin-negative pattern. Although genetic males, possessing a normal set of 44 autosomes and an XY sex chromosome pair, these individuals appear as normal females whose condition is often not recognized until puberty.[87, 88] Although there is normal female breast development, menstruation does not occur and pubic and axillary hair is sparse. The vaginal and urethral openings are normally located. There is, however, no uterus. Testes may be located intra- or extra-abdominally.[51, 89]

Several studies have indicated a high familial incidence.[51, 90, 91] The most acceptable interpretation is that the condition is either a sex-linked recessive or a sex-limited autosomal dominant.[51, 90] The mutant gene is transmitted by phenotypically normal heterozygous females to half of their daughters, who become carriers, and half of their sons. Male offspring who carry the gene develop the syndrome and appear to be females, while those who do not are normal males and should reproduce normally.

4. Triplo-X syndrome. This new clinical entity has been described in at least 7 published cases.[92-94] Since all were found by surveying mentally defective patients, it is not surprising that mental retardation appears to be a constant feature. This emerges as the only constant finding, although other anomalies may be present. These women are phenotypic females and may reproduce normally.

The chromosome number is 47, 22 pairs of autosomes plus 3 X-chromosomes. One characteristic feature of great interest is the finding of an increased number of chromatin-positive cells from buccal smears, when compared with those of XX individuals. In addition, many of these cells have 2 sex chromatin bodies. This unusual pattern makes feasible the detection of these individuals by surveying buccal smears since, with 3 possible exceptions, this finding appears only in triplo-X females.

The exceptions are important in connec-

tion with the problem of the origin of sex chromatin since an extra X-chromosome correlated with an additional sex chromatin body in the triplo-X syndrome is in accord with the single-X theory of the sex chromatin mass. Both de Carli and co-workers[95] and Sandberg, Crosswhite, and Gordy[96] have published reports of mentally retarded females trisomic for a chromosome in the 6-12 group. The sex chromosome complement was originally thought to be XX. However, buccal smears from both these women were indistinguishable from those of triplo-X individuals. Because the triplo-X syndrome is not well defined clinically, and because the chromosomes in the 6-12 group are distinguished from the X only with great difficulty, it is quite likely that these cases are examples of the triplo-X syndrome.[97]

As previously discussed, 2 cases of XXXY Klinefelter's syndrome have been noted. Cells from buccal smears were observed with double chromatin bodies.[63]

The third exception, described by Fraccaro, Kaijser, and Lindsten[98] and discussed more fully in the section on congenital anomalies, involves a child with 49 chromosomes. The presence of 3 sex chromatin bodies in cells of the buccal epithelium has led to the tentative interpretation of this as an XXXXY individual.[99]

In the normal diploid cell, a single X remains isopyknotic to the autosomes. When additional X-chromosomes are present, as in the above cases, all but one X becomes heteropyknotic during interphase. Thus, the number of sex chromatin bodies is one less than the number of X-chromosomes present in the cell. This dynamic relationship has been suggested in an editorial in *Lancet*.[100]

The term superfemale, originally applied to the triplo-X syndrome by Jacobs, was derived from an analogous situation in *D. melanogaster*.[43] The term triplo-X syndrome seems preferable for clinical medicine.

5. Other developmental sexual anomalies.

A. True hermaphrodites, individuals with both ovarian and testicular tissue, have been studied in relatively few cases. Six instances of a normal female complement have been reported in detail.[101-105] Two of them, however, showed an appreciable number of cells with significant variations.[103] One exhibited some cells with an extra chromosome that may have been a third X, and one showed a few cells with an additional small chromosomal fragment.

A few cases appear to bear a definite relationship to mosaicism. The most enlightening situation, described by Hirschhorn and associates, was presented by a 3-month-old infant with a phallus and separate urethral and vaginal perineal openings. Internally, there were a uterus, Fallopian tubes, and 2 gonads, in the position of ovaries, which showed both ovarian and testicular components. Two types of cells were present in the bone marrow in approximately equal numbers; one type had 44 autosomes and a single X; the other, 44 autosomes and an X and a Y. Cells from skin cultures were found to have 44 autosomes plus an X and a Y. The XO tissue may have arisen as a result of mitotic nondisjunction in the developing mesoderm.

Miller[106] has observed 2 instances of a somewhat similar situation. In one of these, there appears to be a difference between the right and left sides of the genital tract which may be correlated with an XO/XY mosaicism in the local connective tissue as well as in the blood.

Generalizations are not yet possible. It seems clear that hermaphroditism represents a heterogeneous group of conditions, some of which may be clarified by studies correlating clinical findings and chromosome analyses from a variety of tissues, with care to uncover the possible role of mosaicism.

B. A striking example of mosaicism was illustrated by Jacobs and colleagues[45] in a 52-year-old phenotypic female. This mentally retarded individual had a masculine upper trunk and shoulder girdle, no breast development, but the pubic hair of a normal female. External genitals were underdeveloped, except for a normal clitoris. No vagina was present and no uterus was palpable.

Smears from the buccal mucosa revealed

the type of pattern seen in the triplo-X female, whereas the blood film revealed polymorphonuclear leukocytes without drumsticks, as seen in normal males.

Chromosomes from a number of different tissues were examined and 2 types of cells could be distinguished in tissue culture, those with 45 chromosomes and those with 47 chromosomes. The former consistently showed only a single X-chromosome and were interpreted as XO, whereas the latter were seen to be XXX-containing cells. The figure 47 predominated in skin cultures from both sides of the body, whereas the bone marrow cultures had only 45 chromosomes.

The most compelling interpretation of these findings is that this individual represents an example of mitotic nondisjunction which probably occurred during the first cleavage division of the fertilized ovum.

C. Rare sex-linked recessive traits which unexpectedly appear in phenotypic females may be clarified by chromosome studies. Nilsson and co-authors[107] described a female child with typical clinical manifestations of severe hemophilia A. This sex-linked recessive trait is expected to occur with a frequency of 1 in 100,000,000 female births, in contrast to a frequency of 1 in 10,000 male births. Analysis of the family pedigree together with quantitative determinations of the antihemophiliac globulin levels in the propositus, her mother, and a heterozygous aunt were as would be expected if the child had been a male. This unusual occurrence was explained by the finding of a negative sex chromatin pattern in the neutrophils and skin. Detailed examination of the chromosomes revealed a normal male complement.

The same authors mention analogous situations. Two cases of typical hemophilia in girls which could be explained in the above fashion were discussed. Walton,[108] in a study of sex-linked muscular dystrophy of the Duchenne type, reported upon one affected girl, in a family including many affected males, who was chromatin negative.

These cases are explainable on the basis that the phenotypic females involved were actually genetic males. However, the explanation for a chromosomal male developing into a normal-appearing female is not readily apparent. On the basis of present knowledge, it must be assumed that these represented cases of the syndrome of testicular feminization or some variant of gonadal dysgenesis.

B. Autosomes.

1. Mongolism. For the pediatrician, the association of an extra chromosome with most cases of Mongolism has been one of the most dramatic medical discoveries in recent years. This was first reported from France by Lejeune and co-workers and shortly thereafter confirmed by others.[109-113] In all these cases, Mongolism was found associated with trisomy for the small satellited autosome 21. Consequently, concepts of the etiology of Mongolism have changed to accomodate this fact.[114] Nondisjunction during meiosis is regarded as the most probable basis for the trisomy. In view of the association of Mongolism with advanced maternal age, the error is thought to be one of oögenesis rather than spermatogenesis.[62]

Exceptions to the association of an extra chromosome 21 and Mongolism have been recorded, indicating that from a cytogenetic point of view, there are at least 2 forms of Mongolism.

Although the risk of producing a Mongoloid child increases with advancing maternal age, familial cases are found in mothers whose age is significantly lower than the mean for all mothers of Mongols.[115] This observation suggests that there may be a group of Mongols in whom maternal age is not an etiological factor. Polani and associates,[116] in examining the chromosomes of 3 Mongols born to young mothers, found one case in which 46 chromosomes were present. Penrose, Ellis, and Delhanty[117] and Carter and his collaborators[118] have reported similar findings.

In these cases, a normal pair of chromosomes 21 has been present in the normal complement of 46 chromosomes. However, not all the autosomes could be paired exactly. One acrocentric chromosome in each

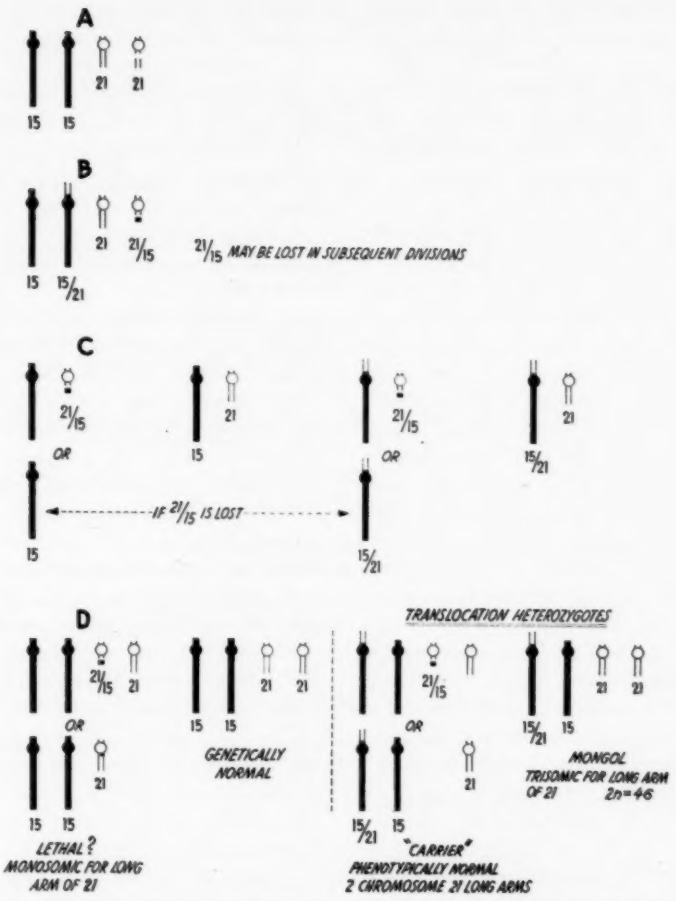

Fig. 4. Mechanism of translocation Mongolism: *A*, Breakage distal to kinetochore in one of pair 15, breakage proximal to kinetochore in one of pair 21. *B*, Reciprocal translocation of chromosomal material. *C*, One of each pair enters gamete (egg); possible combinations illustrated. *D*, Possible zygotes following fertilization by normal sperm. (From Carter, C. O.: Lancet 2:680, 1960.)

case was missing and an extra metacentric was present. The most logical interpretation, first suggested by Polani, is that the unpaired metacentric chromosome is the product of a translocation and represents the fusion of a satellited acrocentric chromosome with the major portion of an additional chromosome 21. In effect, this is equivalent to trisomy for chromosome 21 and represents a mechanism whereby familial cases of Mongolism may be explained. While it is possible that some individuals are more likely to produce nondisjunctional gametes with unbalanced chromosome sets, either because of genetic or environmental factors, it seems more likely that familial cases of Mongolism are due to translocations.

The mechanism outlined in Fig. 4 is based on theoretical considerations and on an analysis of 2 families,[116-117] in which women with 45 chromosomes gave birth to Mongols. Only a few cases of familial Mongols have

Table III. Autosomal aberrations and clinical syndromes

	Chromosome complement	*Abnormality*	*Chromosome involved*	*Clinical findings*
A	47	Trisomy	21	Mongolism[109-113]
	46	Translocation	21-22, 21-(13-15)	Mongolism[115-117]
B	45	Translocation	22-(13-15)	Mental retardation, dwarfism, multiple osseous malformations[124]
C	47	Trisomy	13-15	Cerebral defect, anophthalmia, cleft palate and harelip, polydactyly and trigger thumbs, capillary hemangioma, and heart defect[125]
D	47	Trisomy	17	Dwarfism, webbed neck, micrognathia, odd-shaped head with low-set ears, webbed fingers and toes, hypermobility of shoulders, shield chest, intraventricular defect[126]
E	47	Trisomy	18	Retardation, micrognathia, low-set ears, finger deformities, intraventricular defect[127]
F	47	Trisomy	19	Normal 58-year-old male, father of Mongoloid child[119]
G	47	Trisomy	22	Sturge-Weber syndrome (not all cases)[128-129]
H	47	Enlarged satellites	13-15 21	Marfanlike syndrome[18] Nonspecific congenital anomalies[131]
I	69/46	Triploid skin/diploid marrow		Mental retardation, porencephaly, micrognathia, syndactyly, localized lipomatoses on extremities[38, 39]
Questionable interpretation				
J	47	Trisomy	X or (6-12)*	Mental retardation (triplo-X variant?)[95, 97]
K	49	Double trisomy	(6-12)† or multiple X	Mental retardation, peculiar facies, protruding abdomen, heart and kidney defects, divided scrotum, penis[98, 99]

*Extra chromosome of group indicated.
†More than one extra chromosome of group indicated.

been analyzed for karyotype, and other mechanisms may yet be uncovered. Findings indicate that useful predictions about future pregnancies in younger women who have borne Mongols will have to be based on chromosome analyses. If, as diagrammed, all ova are equally viable, a woman carrying such a translocation, though normal herself, could anticipate that 1 pregnancy in 4 may result in a Mongol child, and 1 pregnancy in 4 may result in abortion; thus only half of her pregnancies would produce normal children. Moreover, half of her normal children would be carriers of the translocation. In essence, a pseudorecessive type of inheritance may exist in these instances. It is now important to determine the relative frequency of "translocation Mongolism" in the population.

The story is not yet complete. Fraccaro, Kaijser, and Lindsten[119] have studied a Mongoloid child with 46 chromosomes who was monosomic for autosome 21 but possessed an additional autosome resembling 19. This was initially presumed to represent an isochromosome resulting from the fusion of the long arms of 2 chromosomes 21. The father, who appeared normal, was found on analysis to have 47 chromosomes, trisomic for 19. Interpretations at this point are difficult.

There have been 3 reports of the association within one individual of Mongolism and Klinefelter's syndrome. In all these cases of double trisomy, an extra X and chromosome 21 were present. The chromosome number was 48.[120-122] In a series of 10 cases of true Klinefelter's syndrome, 2 individuals with

siblings who were Mongoloid were found.[123] This may be a fortuitous association. On the other hand, these cases may indicate that where a tendency for nondisjunction exists the sex chromosomes and chromosome 21 are the ones most likely to be involved.

2. Other congenital anomalies. A number of congenital anomalies associated with changes in karyotype involving autosomes are summarized in Table III.

A. Mongolism.

B. This "polydyspondylism" is illustrated by one individual, a mentally retarded child with multiple osseous malformations, in whom only 45 separate chromosomes could be counted.[124] Analysis led to the interpretation that one unpaired medium-sized autosome represented a reciprocal translocation between a chromosome 22 and one chromosome in the 13-15 group. A very small autosome representing the fused minute short arms of the 2 acrocentric chromosomes was anticipated, but such a fragment had apparently been eliminated during cell division. In such a situation, developmental anomalies presumably result from position effect, deletion, or both phenomena.

C. Two cases of severely stigmatized female infants whose bone marrow cultures showed 47 chromosomes, with trisomy for one of the autosomes in the 13-15 group, have been described.[125]

D. A female infant who died at the age of 5 months showed 47 chromosomes in cultures from skin and muscle with trisomy for chromosome 17.[126] The authors point out similarities to some cases described as the Bonnevie-Ullrich syndrome. Jaundice, which was also present in this case, was presumably due to neonatal hepatitis.

E. Six children clinically resembling the above case who were trisomic for 18 have been noted. In 4 of these cases, the maternal age was over 35.[127]

F. A phenotypically normal male with 47 chromosomes, trisomic for 19, has been discovered. He was the father of an unusual Mongol child.[119] This is an important finding to consider when correlating unusual karyotypes with clinical syndromes.

G. A 4-year-old mentally retarded boy with Sturge-Weber's syndrome has been found to be trisomic for chromosome 22.[128] On the other hand, there has been a report of 2 cases of this syndrome with a normal chromosome complement.[129] The possibility thus exists that this association is fortuitous.

H. Three individuals with Marfan's syndrome who possessed 46 chromosomes have been described.[18] One child with a normal karyotype had ectopia lentis and arthrogrypotic changes. There were no similarly affected relatives. In the other 2 cases, unrelated to each other, similarly afflicted individuals were present in the respective families, in which the condition was inherited as a dominant. Both exhibited the body build of Marfan's syndrome but not ectopia lentis. Their cells in culture showed enlargement of the satellite in one member of a chromosome pair. In one patient, chromosome 13 was involved; in the other individual, chromosome 21. Enlarged satellites are usually not seen to the extent described; this abnormal finding possibly bears a causal relationship to the syndrome. Such a cytogenetic change is due to an increased amount of genetic material which theoretically may occur as a result of a duplication or a translocation. McKusick,[130] however, questions the diagnosis in these 2 cases with enlarged satellites. It is likely that this finding will not be specific for a particular syndrome, inasmuch as there is a description of a chromosome 21 with an enlarged satellite associated with an entirely different spectrum of congenital malformations.[131]

I. One case of a malformation syndrome associated with triploidy exists.[38, 39] Sixty-nine chromosomes, interpreted as 3 haploid sets of autosomes with an XXY, were present in skin cultures. However, bone marrow cells showed a normal diploid set of chromosomes. This individual represents an unusual form of mosaicism.

J. Two possible trisomic syndromes which may really be variants of the triplo-X syndrome have been discussed.[95, 96] Both indi-

viduals were mentally retarded but neither presented a characteristic clinical appearance.

K. Fraccaro and collaborators[98, 99] have described a child with 49 chromosomes whose interpretation presents a similar problem. This 7-year-old retarded boy yielded cells from both bone marrow and skin cultures with 49 chromosomes. At first, trisomy for chromosomes 8 and 11 was suspected, along with a sex complement of XXY. Since buccal smears revealed some cells with 3 nuclear chromatin bodies, the alternative possibility exists of a normal assortment of autosomes with an XXXXY sex chromosome arrangement. If so, the extra X-chromosomes exhibit a variable size which may be due to different degrees of heteropyknosis.

3. Chronic myeloid leukemia. The first unequivocal association of a chromosomal anomaly with a malignant disease has been described for chronic myeloid leukemia.[132, 133] It was noted by both Nowell and Hungerford and Baikie and his associates that while the chromosome number was normal, one of the smallest acrocentric chromosomes was abnormally small. Finally, in a more extensive study from the Western General Hospital in Edinburgh, Tough and co-authors[134] suggest that the chromosome involved in Mongolism may be the same as that which appears abnormal in chronic myeloid leukemia. The symbol Ph^1 was chosen to identify the chromosome as it indicates the location of the laboratories, Philadelphia, where Nowell and Hungerford first noted its occurrence. Apparently, the Ph^1 chromosome is derived from either 21 or 22 by deletion of a portion of its long arm or by means of a translocation. The significance of this association, however, is not understood.

DISCUSSION

It is possible at present to discuss a few broad generalizations that have emerged from the large number of observations presented within the field of human cytogenetics.

Sex determination in humans is now better understood. Previously the model for the mechanism of sex determination was based on studies in *D. melanogaster*. In this organism XO individuals are male and hence the Y-chromosome is not a male-determining body; phenotypic sex is the result of a balance between the number of X-chromosomes in relation to the number of sets of autosomes. With the discovery of XO females in *Mus musculus*[135] and man and the elucidation of the sex chromosomal arrangement in Klinefelter's syndrome and the syndrome of testicular feminization, it seemed certain that the Y-chromosome in mammals is strongly male determining. This is a valid generalization except as applied to hermaphrodites. It is tempting to speculate that mosaicism will be an important factor in the etiology of these individuals but at present they represent the major exception to the rule that the Y-chromosome is always associated with a testis or lack of a gonad, never an ovary.

The Y-chromosome is probably important in other aspects of development since the XO state is commonly associated with a variety of anomalies. Similar anomalies, however, may be present in association with the Y-chromosome, as in males with so-called Turner's syndrome. Little else is known about the role of the Y-chromosome. In humans, it is thought to be entirely heterochromatic; with the possible exception of the trait for hairy ears, no Mendelizing genes have been unequivocally demonstrated to be present on it.[136, 137] Possibly its role is more regulatory in nature. It is the only chromosome known which is not necessary for viability, and comparative studies on bone age indicate a possible inhibitory effect on skeletal maturation, since XX and XO individuals have a more advanced skeletal age than XY or XXY individuals.[138]

The X-chromosome has many indispensible functions. In addition to its role in sex determination, which cannot be precisely defined, over 40 different clinical syndromes due to single gene defects on the X are known.[139] It is clear that nullo-X conditions (YY, YO, or OO) are inviable. It is undoubtedly significant that so many anomalous sex chromosomal conditions have already

been described in contrast to autosomal syndromes. This may reflect a greater frequency of nondisjunction with respect to the sex chromosomes, or their heterochromatic nature may permit the existence of more viable anomalies.

No generalizations concerning the role of individual autosomes in development are yet permissible except that the loss of an autosome or any large portion thereof is likely to be inviable. The clinical syndromes associated with additional chromosomal material are of a generalized nature. This is exemplified by Mongolism, with its array of defects, and Klinefelter's syndrome where, although the major pathologic findings are associated with the reproductive system, there is an increased incidence of mental retardation.

The occurrence of nondisjunction in humans has not been observed; it can only be deduced from the existence of trisomic and monosomic karyotypes. The fact that maternal age but not paternal age is advanced in Mongolism suggests that nondisjunction is more likely during oögenesis than spermatogenesis. Maternal age is also advanced in Klinefelter's syndrome but no statements regarding paternal age can be made. A consideration of pedigrees where Klinefelter's syndrome and red-green color blindness coexist, can provide an insight into this problem. The data available indicate that nondisjunction may occur during oögenesis but is insufficient to confirm or refute its occurrence during spermatogenesis.[140] For Turner's syndrome, maternal age approximates that of normal controls, but we have seen no data concerning paternal age. Studies of color-blind individuals with Turner's syndrome indicate that if nondisjunction is the correct etiological basis, it may occur during either oögenesis or spermatogenesis.[141, 142] Still to be explained is the lower incidence of Turner's syndrome relative to Klinefelter's since theoretically nondisjunction should produce more XO than XXY individuals. Moore's data indicate that the latter syndrome is far more common.[56] Possibly differential viability in the abnormal gametes or developing fetuses could account for this discrepancy but we are still left with the inconsistency in the role of maternal age.

The basis for the occurrence of translocations is not understood; the chromosomes involved in translocations have, in cases thus far reported, been satellited and thus presumably take part in nucleolus formation. Ferguson-Smith and Handmaker[18] suggest that the characteristic association they have described may be connected with breakage of the chromosomes involved. The probability of reciprocal translocation between satellited chromosomes would, therefore, be greater than between nonsatellited chromosomes.

Finally, it is worth noting that chromosomal changes are most likely to be associated with those syndromes characterized by severe generalized congenital anomalies, with primary gonadal disorders, or with a dominant mode of inheritance.

SUMMARY

The following are the most significant points to emerge in summarizing the present status of the field of human cytogenetics.

1. The human chromosome number is 46; a standard system of nomenclature has been established for identification of somatic metaphase chromosomes. Methods, however, are not yet refined enough to detect small changes within individual chromosomes.

2. The sexual dimorphism existing in somatic cells due to the presence of sex chromatin in nuclei of females permits a rapid survey method for detecting discrepancies between phenotypic and genotypic sex. Chromatin positivity or negativity, however, should be correlated with chromosome analyses since only limited deductions concerning the sex chromosome complement can be made from the presence or absence of sex chromatin.

3. The sex chromatin body in female diploid cells is a manifestation of a single X-chromosome. A cell with 2 sets of autosomes and 2 X-chromosomes is found to have

one heteropyknotic X during interphase. The XO or XY cell has no heteropyknotic X. In cells with 2 sets of autosomes and more than 2 X-chromosomes, more than one X may be heteropyknotic, permitting a single X to remain isopyknotic to the autosomes.

4. The greater number of chromosomal anomalies have been found to involve the sex chromosomes. This may reflect the heterochromatic nature of the X and Y, permitting unbalanced conditions to be viable for the organism. Only one monosomic condition, the XO, has thus far been described. No trisomic conditions for the larger autosomes have been detected.

5. The Y-chromosome is a male-determining body. The only cases in which a testis is present in the absence of a Y concern hermaphrodites. In other situations, the Y in combination with one or more X's influences development in a male direction. The female-determining potentialities of the X are not so clearly delineated as the wide range of phenotypes associated with the XO condition seems to indicate. The association of mental retardation with anomalies of the X-chromosome is unusually prevalent.

6. Clinical conditions characterized by primary gonadal failure or intersexuality have been clarified by chromosomal analyses. There is need for detailed studies of cells from many tissues to delineate the role of mosaicism, especially as it may pertain to hermaphroditism.

7. Nondisjunction, influenced by maternal age, is an important etiological basis for the existence of trisomic states.

8. For the pediatrician, the studies on Mongolism are of primary importance. The genetic aspects of the condition have been clarified and a basis for the familial occurrence emphasizes the necessity of chromosomal analyses in providing information in regard to future pregnancies in young mothers with affected children.

9. The presence of an attenuated chromosome 21 or 22, the Ph^1 chromosome, is associated with chronic myeloid leukemia.

REFERENCES

1. Barr, M. L., and Bertram, L. F.: A Morphological Distinction Between Neurones of the Male and Female and the Behavior of the Nucleolar Satellite During Accelerated Nucleoprotein Synthesis, Nature **163:** 676, 1949.
2. Tjio, J. H., and Levan, A.: The Chromosome Number of Man, Hereditas **42:** 1, 1956.
3. Barr, M. L.: Sex Chromatin and Phenotype in Man, Science **130:** 679, 1959.
4. Barr, M. L.: An Interim Note on the Application of the Skin Biopsy Test of Chromosomal Sex to Hermaphrodites, Surg. Gynec. & Obst. **99:** 184, 1954.
5. Bradbury, J. T., Bunge, R. G., and Boceabella, R. A.: Chromatin Test in Klinefelter's Syndrome, J. Clin. Endocrinol. **16:** 689, 1956.
6. Plunkett, E. R., and Barr, M. L.: Testicular Dysgenesis Affecting Seminiferous Tubules, Principally With Chromatin Positive Nuclei, Lancet **2:** 853, 1956.
7. Decourt, L., Sasso, W., Chiorboli, E., and Fernandes, J. M.: Sobre o sexo genetico nas parientes con sindro, e de Turner, Rev. Assoc. med. brasil. **1:** 203, 1954.
8. Polani, P. E., Hunter, W. E., and Lennox, B.: Chromosomal Sex in Turner's Syndrome With Coarctation of the Aorta, Lancet **2:** 120, 1954.
9. Wilkins, L., Grumbach, M. M., and Van Wyk, J. J.: Chromosomal Sex in Ovarian Agenesis, J. Clin. Endocrinol. **14:** 1270, 1954.
10. Nelson, W. O.: Reproduction and Infertility, Michigan State University Centennial Symposium Report, East Lansing, Mich., 1955, p. 79.
11. Ford, C. E., and Hamerton, J. L.: The Chromosomes of Man, Nature **178:** 1020, 1956.
12. Ford, C. E., Jacobs, P. A., and Lajtha, L. G.: Human Somatic Chromosomes, Nature **181:** 1565, 1958.
13. Chu, E. H. Y., and Giles, N. H.: Human Chromosome Complements in Normal Somatic Cells in Culture, Am. J. Human Genet. **11:** 63, 1959.
14. Stern, C.: The Chromosomes of Man, Am. J. Human Genet. **11:** 301, 1959.
15. Moorehead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M., and Hungerford, D. A.: Chromosome Preparations of Leukocytes Cultured From Human Peripheral Blood, Exper. Cell Res. **20:** 613, 1960.
16. Report of a Study Group: A Proposed Standard System of Nomenclature of Human Mitotic Chromosomes, Am. J. Human Genet. **12:** 384, 1960.
17. Patau, K.: The Identification of Individual Chromosomes, Especially in Man, Am. J. Human Genet. **12:** 250, 1960.
18. Ferguson-Smith, M. A., and Handmaker, S.

D.: Observations on the Satellited Human Chromosomes, Lancet 1: 638, 1961.
19. Hannah, Aloha: Localization and Function of Heterochromatin in *Drosophila melanogaster,* Advances in Genetics, vol. 4, New York, 1951, Academic Press, Inc.
20. Heitz, E.: Das Heterochromatin der Moose. I., Jahrb. wissensch. Bot. 69: 762, 1928.
21. White, M. J. D.: Animal Cytology and Evolution, ed. 2, London, 1954, Cambridge University Press.
22. Heitz, E.: Die somatische Heteropyknose bei *Drosophila melanogaster* und ihre genetische Bedeutung, Ztschr. Zellforsch. u. mikroskop. Anat. 20: 237, 1933.
23. Ohno, S., Kaplan, W. D., and Kinosita, R.: Heterochromatic Regions and Nucleolus Organizers in Chromosomes of the Mouse, *Mus musculus,* Exper. Cell Res. 13: 358, 1957.
24. Ohno, S., Kaplan, W. D., and Kinosita, R.: The Centromeric and Nucleolus-Associated Heterochromatin of *Rattus norvegicus,* Exper. Cell Res. 16: 348, 1959.
25. Sachs, Leo: Sex-Linkage and the Sex Chromosomes in Man, Ann. Eugenics 18: 255, 1954.
26. Schultz, J.: The Function of Heterochromatin, Proc. Seventh Internat. Congr. Genet., 1939 (suppl. vol. to J. Genet., Cambridge, 1941).
27. Russell, L. B., and Bangham, J. W.: Further Analysis of Variegated-Type Position Effects From X-Autosome Translocations in the Mouse, Genetics 45: 1008, 1960.
28. Ohno, S., Kaplan, W. D., and Kinosita, R.: Formation of the Sex Chromatin by a Single X-Chromosome in Liver Cells of *Rattus norvegicus,* Exper. Cell Res. 18: 415, 1959.
29. Ohno, S., Kaplan, W. D., and Kinosita, R.: On the Isopycnotic Behavior of the XX-Bivalent in Oöcytes of *Rattus norvegicus,* Exper. Cell Res. 19: 637, 1960.
30. Ohno, S., Kaplan, W. D., and Kinosita, R.: On the End-to-End Association of the X- and Y-Chromosomes of *Mus musculus,* Exper. Cell Res. 18: 282, 1959.
31. Moore, K. L., Graham, M. A., and Barr, M. L.: The Detection of Chromosomal Sex in Hermaphrodites From a Skin Biopsy, Surg. Gynec. & Obst. 96: 641, 1953.
32. Moore, K. L., and Barr, M. L.: Smears From the Oral Mucosa in the Detection of Chromosomal Sex, Lancet 2: 57, 1955.
33. Davidson, W. M., and Smith, D. R.: A Morphological Sex Difference in the Polymorphonuclear Neutrophil Leucocytes, Brit. M. J. 2: 6, 1954.
34. Ohno, S., Kaplan, W. D., and Kinosita, R.: Basis of Nuclear Sex Difference in Somatic Cells of the Opossum, *Didelphis virginiana,* Exper. Cell Res. 19: 417, 1960.
35. Ohno, S., Kaplan, W. D., and Kinosita, R.: On the Sex Chromatin of *Gallus domesticus,* Exper. Cell Res. 19: 180, 1960.
36. Atkin, N. B.: A Single Heteropycnotic Chromosome in a Human Tumor, Exper. Cell Res. 20: 214, 1960.
37. Ohno, S., and Makino, S.: The Single-X Nature of Sex Chromatin in Man, Lancet 1: 78, 1961.
38. Böök, J. A., and Santesson, B.: Malformation Syndrome in Man Associated With Triploidy (69 Chromosomes), Lancet 1: 858, 1960.
39. Böök, J. A., and Santesson, B.: Further Studies in Clinical Cytogenetics, Acta Univ. Lund, ser. 2, 56: 19, 1960.
40. Lejeune, J., Turpin, R., and Decourt, J.: Aberrations chromosomiques et maladies humaines. Syndrome de Klinefelter XXY à 46 chromosomes par fusion centromérique T-T, Compt. rend. Acad. sc. 250: 2468, 1960.
41. Lewis, E. B.: The Phenomenon of Position Effect, Advances in Genetics, vol. 3, New York, 1950, Academic Press, Inc.
42. Bridges, C. B.: Nondisjunction as Proof of the Chromosome Theory of Inheritance, Genetics 1: 1, 1916.
43. Bridges, C. B.: Sex in Relation to Chromosomes and Genes, Am. Nat. 59: 127, 1925.
44. Mottram, J. C.: The Effect of Carbon Dioxide on the Occurrence of Nondisjunction in *Drosophila,* J. Exper. Biol. 7: 370, 1930.
45. Jacobs, P. A., Harnden, D. G., Court Brown, W. M., Goldstein, J., Close, H. G., MacGregor, T. N., Maclean, N., and Strong, J. A.: Abnormalities Involving the X-Chromosome in Women, Lancet 1: 1213, 1960.
46. Hirschhorn, K., Decker, W. H., and Cooper, H. L.: Human Intersex With Chromosome Mosaicism of Type XY/XO, New England J. Med. 263: 1044, 1960.
47. Court Brown, W. M., Jacobs, P. A., and Doll, R.: Interpretation of Chromosome Counts Made on Bone Marrow, Lancet 1: 160, 1960.
48. Klinefelter, J. R., Jr., Reifenstein, E. C., Jr., and Albright, F.: Syndrome Characterized by Gynecomastia, Aspermatogenesis Without A-Leydigism, and Increased Excretion of Follicle-Stimulating Hormone, J. Clin. Endocrinol. 2: 615, 1942.
49. Heller, C. G., and Nelson, W. O.: Hyalinization of the Seminiferous Tubules Associated With Normal or Failing Leydig-Cell Function: Discussion of Relationship to Eunuchoidism, Gynecomastia, Elevated Gonadotrophins, Depressed 17-Ketosteroids, and Estrogens, J. Clin. Endocrinol. 5: 1, 1945.
50. Stewart, J. S. S., Mack, W. S., Govan, A. D. T., Ferguson-Smith, M. A., and Lennox, B.: Klinefelter's Syndrome: Clinical and Hormonal Aspects, Quart. J. Med. 28: 561, 1959.
51. Grumbach, M. M., and Barr, M. L.: Cytologic Tests of Chromosomal Sex in Relation to Sexual Anomalies in Man, Recent Progr. Hormone Res. 14: 255, 1958.
52. Ferguson-Smith, M. A.: Chromatin-Positive

Klinefelter's Syndrome (Primary Microorchidism) in a Mental Deficiency Hospital, Lancet **1:** 928, 1958.
53. Ford, C. E.: Human Cytogenetics, Am. J. Human Genet. **12:** 104, 1960.
54. Pasqualini, R. Q., Vidal, G., and Bur, G. E.: Psychopathology of Klinefelter's Syndrome, Lancet **2:** 164, 1957.
55. Ferguson-Smith, M. A.: The Prepuberal Testicular Lesion in Chromatin-Positive Klinefelter's Syndrome as Seen in Mentally-Handicapped Children, Lancet **1:** 219, 1959.
56. Moore, K. L.: Sex Reversal in Newborn Babies, Lancet **1:** 217, 1959.
57. How Common is Sex Reversal? (editorial), Lancet **1:** 237, 1959.
58. Jacobs, P. A., and Strong, J. A.: A Case of Human Intersexuality Having a Possible XXY Sex-Determining Mechanism, Nature **183:** 302, 1959.
59. Ford, C. E., Polani, P. E., Briggs, J. H., and Bishop, P. M. F.: A Presumptive Human XXY/XX Mosaic, Nature **183:** 1030, 1959.
60. Ferguson-Smith, M. A.: Cytogenetics in Man, A. M. A. Arch. Int. Med. **105:** 627, 1960.
61. Lenz, W., Nowakowski, H., Prader, A., and Schirren, C.: Die Aetiologie des Klinefelter-Syndroms, Schweiz. med. Wchnschr. **89:** 727, 1959.
62. Penrose, L. S.: Mongolian Idiocy (Mongolism) and Maternal Age, Ann. New York Acad. Sc. **57:** 494, 1954.
63. Ferguson-Smith, M. A., Johnston, A. W., and Handmaker, S. D.: Primary Amentia and Micro-orchidism Associated With an XXXY Sex Chromosome Constitution, Lancet **2:** 184, 1960.
64. Muldal, S., and Ockey, C. R.: The "Double Male": A New Chromosome Constitution in Klinefelter's Syndrome, Lancet **2:** 492, 1960.
65. Stewart, J. S. S., Ferguson-Smith, M. A., Lennox, B., and Mack, W. S.: Klinefelter's Syndrome: Genetic Studies, Lancet **2:** 117, 1958.
66. Bassöe, H. H., Fuchs, F., and Riis, P.: Nuclear Sex in Familial Gonadal Dysgenesis, Acta endocrinol. **28:** 389, 1958.
67. Nadler, C. S., Steiger, W. A., Troncelleti, M., and Durrant, T. M.: Dystrophica Myotonica With Special Reference to Endocrine Function (Klinefelter's Syndrome), J. Clin. Endocrinol. **10:** 630, 1950.
68. Sohval, A. R., Gabrilove, J. L., Gaines, J. A., and Soffer, L. J.: Observations on the Sex Chromatin in Testicular Disorders, J. Mt. Sinai Hosp., New York **23:** 647, 1956.
69. Turner, H. H.: A Syndrome of Infantilism, Congenital Webbed Neck and Cubitus Valgus, Endocrinology **23:** 566, 1938.
70. Haddad, H. M., and Wilkins, L.: Congenital Anomalies Associated With Gonadal Aplasia: Review of 55 Cases, Pediatrics **23:** 885, 1959.
71. Jost, A.: Recherches sur la différentiation sexuelle de l'embryon de lapin: III. Role des gonads foetales dans la différentiation sexuelle somatique, Arch. anat. microbiol. et morphol. exper. **36:** 271, 1947.
72. Ford, C. E., Jones, K. W., Polani, P. E., Almeida, J. C., and Briggs, J. H.: A Sex Chromosome Anomaly in a Case of Gonadal Dysgenesis (Turner's Syndrome), Lancet **1:** 711, 1959.
73. Tjio, J. H., Puck, T. T., and Robinson, A.: The Somatic Chromosome Constitution of Some Human Subjects With Genetic Defects, Proc. Nat. Acad. Sc. **45:** 1008, 1959.
74. Fraccaro, M., Kaijser, K., and Lindsten, J.: Chromosome Complement in Gonadal Dysgenesis (Turner's Syndrome), Lancet **1:** 886, 1959.
75. Stewart, J. S. S.: Gonadal Dysgenesis: the Genetic Significance of Unusual Variants, Acta endocrinol. **33:** 89, 1960.
76. Bahner, F., Schwarz, G., Harnden, D. G., Jacobs, P. A., Hienz, H. A., and Walter, K.: A Fertile Female With XO Sex Chromosome Constitution, Lancet **2:** 100, 1960.
77. Steiker, D. D., Mellman, W. J., Bongiovanni, A. M., Eberlein, W. R., and Leboeuf, G.: Turner's Syndrome in the Male, J. PEDIAT. **58:** 321, 1961.
78. Bloise, W., Bottura, C., Assis, L. M. de, and Ferrari, I.: Gonadal Dysgenesis (Turner's Syndrome) With Male Phenotype and XO Chromosomal Constitution, Lancet **2:** 1059, 1960.
79. Grumbach, M. M., Morishima, A., and Chu, E. H. Y.: On the Sex Chromatin and the Sex Chromosomes in Sexual Anomalies in Man: Relation to Origin of the Sex Chromatin, Presented at the thirtieth annual meeting of the Society for Pediatric Research at Swampscott, Mass., May 3 to 4, 1960.
80. Grumbach, M. M.: Personal communication.
81. Fraccaro, M., Ikkos, D., Lindsten, J., Luft, R., and Kaijser, K.: A New Type of Chromosomal Abnormality in Gonadal Dysgenesis, Lancet **2:** 1144, 1960.
82. Harnden, D. G., and Stewart, J. S. S.: The Chromosomes in a Case of Pure Gonadal Dysgenesis, Brit. M. J. **2:** 1285, 1959.
83. Hoffenburg, R., and Jackson, W. P. U.: Gonadal Dysgenesis in Normal-Looking Females, Brit. M. J. **1:** 1281, 1957.
84. Hauser, A., Keller, M., and Wenner, R.: Gonadendysgenesie, Schweiz. med. Wchnschr. **86:** 299, 1956.
85. Greenblatt, R. B., Carmona, N., and Higdon, L.: Gonadal Dysgenesis With Androgenic Manifestations in the Tall Eunuchoid Female, J. Clin. Endocrinol. **16:** 235, 1956.
86. Elliott, G. A., Sandler, A., and Rabinowitz, D.: Gonadal Dysgenesis in Three Sisters, J. Clin. Endocrinol. **19:** 995, 1959.
87. Jacobs, P. A., Baikie, A. G., Court Brown, W. M., Forrest, H., Roy, J. R., Stewart, J. S. S., and Lennox, B.: Chromosomal Sex in

the Syndrome of Testicular Feminization, Lancet **2:** 591, 1959.
88. Puck, T. T.: Cytogenetic Studies on Human Somatic Cells in Vitro, Science **130:** 1423, 1959.
89. Wilkins, L.: The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence, ed. 2, Springfield, Ill., 1957, Charles C Thomas, Publisher.
90. Sternberg, W. N., and Kloepfer, H. W.: Genetic and Pathologic Study of Simulant Females (Testicular Feminization Syndrome), presented at a meeting of the American Society of Human Genetics at Memphis, Tenn., April 28 to 30, 1960.
91. Witschi, E., Nelson, W. O., and Segal, S. J.: Genetic, Developmental, and Hormonal Aspects of Gonadal Dysgenesis and Sex Inversion in Man, J. Clin. Endocrinol. **17:** 737, 1957.
92. Jacobs, P. A., Baikie, A. G., Court Brown, W. M., MacGregor, T. N., Maclean, M., and Harnden, D. G.: Evidence for the Existence of the Human "Super Female," Lancet **2:** 423, 1959.
93. Stewart, J. S. S., and Sanderson, A. R.: Fertility and Oligophrenia in an Apparent Triplo-X Female, Lancet **2:** 21, 1960.
94. Fraser, J. H., Campbell, J., MacGillivray, R. C., Boyd, E., and Lennox, B.: The XXX Syndrome. Frequency Among Mental Defectives and Fertility, Lancet **2:** 626, 1960.
95. Carli, L. de, Nuzzo, F., Chiarelli, B., and Poli, E.: Trisomic Condition of a Large Chromosome in a Woman With Mongoloid Traits, Lancet **2:** 130, 1960.
96. Sandburg, A. A., Crosswhite, L. H., and Gordy, E.: Trisomy of a Large Chromosome. Association With Mental Retardation, J. A. M. A. **174:** 221, 1960.
97. Jacobs, P. A., Harnden, D. G., Court Brown, W. M., and Baikie, A. G.: Trisomic Condition of a Large Chromosome, Lancet **2:** 368, 1960.
98. Fraccaro, M., Kaijser, K., and Lindsten, J.: A Child With 49 Chromosomes, Lancet **2:** 899, 1960.
99. Fraccaro, M., and Lindsten, J.: A Child With 49 Chromosomes, Lancet **2:** 1303, 1960.
100. More Chromosome Anomalies (editorial), Lancet **2:** 191, 1960.
101. Hungerford, D. A., Donnelly, A. J., Nowell, P. C., and Beck, S.: The Chromosome Constitution of a Human Phenotypic Intersex, Am. J. Human Genet. **11:** 215, 1959.
102. Harnden, D. G., and Armstrong, C. N.: The Chromosomes of a True Hermaphrodite, Brit. M. J. **2:** 1287, 1959.
103. Ferguson-Smith, M. A., Johnston, A. W., and Weinberg, A. N.: The Chromosome Complement in True Hermaphroditism, Lancet **2:** 126, 1960.
104. Assis, L. M. de, Epps, D. R., and Bottura, C.: Chromosomal Constitution and Nuclear Sex of a True Hermaphrodite, Lancet **2:** 129, 1960.
105. Sasaki, M., and Makino, S.: The Chromosomal Constitution of a Human Hermaphrodite, Texas Rep. Biol. & Med. **18:** 493, 1960.
106. Miller, O. J.: Personal communication.
107. Nilsson, I. M., Bergman, S., Reitalu, J., and Waldenstrom, J.: Hemophilia A in a "Girl" With Male Sex Chromatin Pattern, Lancet **2:** 264, 1959.
108. Walton, J. N.: The Inheritance of Muscular Dystrophy; Further Observations, Ann. Human Genet. **21:** 40, 1956.
109. Lejeune, J., Gautier, M., and Turpin, R.: Les Chromosomes humains en culture de tissus, Compt. rend. Acad. sc. **248:** 602, 1959.
110. Lejeune, J., Gautier, M., and Turpin, R.: Étude des chromosomes somatiques de neuf enfants mongoliens, Compt. rend. Acad. sc. **248:** 1721, 1959.
111. Lejeune, J., Turpin, R., and Gautier, M.: Le Mongolisme premier example d'abérration autosomique humaine, Ann. génét. **1:** 41, 1959.
112. Jacobs, P. A., Court Brown, W. M., Baikie, A. G., and Strong, J. A.: The Somatic Chromosomes in Mongolism, Lancet **1:** 710, 1960.
113. Böök, J. A., Fraccaro, M., and Lindsten, J.: Cytogenetical Observations in Mongolism, Acta paediat. **48:** 453, 1959.
114. Warkany, J.: Etiology of Mongolism, J. PEDIAT. **56:** 412, 1960.
115. Penrose, L. S.: Maternal Age in Familial Mongolism, J. Ment. Sc. **97:** 738, 1951.
116. Polani, P. E., Briggs, J. H., Ford, C. E., Clarke, C. M., and Berg, J. M.: A Mongol Girl With 46 Chromosomes, Lancet **1:** 721, 1960.
117. Penrose, L. S., Ellis, J. R., and Delhanty, J. D. A.: Chromosomal Translocations in Mongolism and in Normal Relatives, Lancet **2:** 409, 1960.
118. Carter, C. O., Hamerton, J. L., Polani, P. E., Gunalp, A., and Weller, S. D. V.: Chromosome Translocation as a Cause of Familial Mongolism, Lancet **2:** 678, 1960.
119. Fraccaro, M., Kaijser, K., and Lindsten, J.: Chromosomal Abnormalities in Father and Mongol Child, Lancet **1:** 724, 1960.
120. Ford, C. E., Jones, K. W., Miller, O. J., Mittwoch, U., Penrose, L. S., Ridler, M., and Shapiro, A.: The Chromosomes in a Patient Showing Both Mongolism and the Klinefelter Syndrome, Lancet **1:** 709, 1959.
121. Lehmann, O., and Forssman, H.: Klinefelter's Syndrome and Mongolism in the Same Person, Acta pediat. **49:** 536, 1960.
122. Lanman, J. T., Sklarin, B. S., Cooper, H. L., and Hirschhorn, K.: Klinefelter's Syndrome in a Ten-Month-Old Mongolian Idiot: Report of a Case With Chromosome Analysis, New England J. Med. **263:** 887, 1960.

123. Mosier, H. D., Scott, L. W., and Cotter, L. H.: The Frequency of the Positive Sex Chromatin Pattern in Males With Mental Deficiency, Pediatrics **25:** 291, 1960.
124. Turpin, R., Lejeune, J., Lafourcade, J., and Gautier, M.: Abérrations chromosomiques et maladies humaines. La Polydysspondylie à 45 chromosomes. Compt. rend. Acad. sc. **248:** 3636, 1959.
125. Patau, K., Smith, D. W., Therman, E., Inhorn, S. L., and Wagner, H. P.: Multiple Congenital Anomaly Caused by an Extra Autosome, Lancet **1:** 790, 1960.
126. Edwards, J. H., Harnden, D. G., Cameron, A. H., Crosse, M., and Wolff, O. H.: A New Trisomic Syndrome, Lancet **1:** 787, 1960.
127. Smith, D. W., Patau, K., Therman, E., and Inhorn, S. L.: A New Autosomal Trisomy Syndrome: Multiple Congenital Anomalies Caused by an Extra Chromosome, J. Pediat. **57:** 338, 1960.
128. Hayward, M. D., and Bower, B. D.: Chromosomal Trisomy Associated With the Sturge-Weber Syndrome, Lancet **2:** 844, 1960.
129. Lehmann, O., and Forssman, H.: Chromosomes in the Sturge-Weber Syndrome, Lancet **2:** 1450, 1960.
130. McKusick, V. A.: Chromosomes in Marfan's Disease, Lancet **1:** 1194, 1960.
131. Buckton, K. E.: Personal communication.
132. Nowell, P. C., and Hungerford, D. A.: A Minute Chromosome in Human Chronic Granulocytic Leukemia, Science **132:** 1497, 1960.
133. Baikie, A. G., Buckton, K. E., Harnden, D. G., Jacobs, P. A., and Tough, I. M.: A Possible Specific Chromosome Abnormality in Human Chronic Myeloid Leukemia, Nature **188:** 1165, 1960.
134. Tough, I. M., Court Brown, W. M., Baikie, A. G., Buckton, K. E., Harnden, D. G., Jacobs, P., King, M., and McBride, J. A.: Cytogenetic Studies in Chronic Myeloid Leukemia and Acute Leukemia Associated With Mongolism, Lancet **1:** 411, 1961.
135. Welshons, W. J., and Russell, L. B.: The Y-Chromosome as the Bearer of Male-Determining Factors in the Mouse, Proc. Nat. Acad. Sc. **45:** 560, 1959.
136. Gates, R. R.: Y-Chromosome Inheritance of Hairy Ears, Science **132:** 145, 1960.
137. Stern, C.: The Problem of Complete Y-Linkage in Man, Am. J. Human Genet. **9:** 147, 1957.
138. Tanner, J. M., Prader, A., Habich, H., and Ferguson-Smith, M. A.: Genes on the Y-Chromosome Influencing Rate of Maturation in Man, Lancet **2:** 141, 1959.
139. Neel, J. V., and Schull, W. J.: Human Heredity, University of Chicago Press, 1954.
140. Nowakowski, H., Lenz, W., and Parada, J.: Diskrepanz zwischen Chromatinbefund und genetischem Geschlecht beim Klinefelter Syndrom, Acta Endocrinol. **30:** 296, 1959.
141. Polani, P. E., Lessof, M. H., and Bishop, P. M. F.: Colour-Blindness in "Ovarian Agenesis" (Gonadal Dysplasia), Lancet **2:** 118, 1956.
142. Walls, G. L.: Peculiar Color-Blindness in Peculiar People, A. M. A. Arch. Ophth. **62:** 13, 1959.

TROPICAL PEDIATRICS

The state of pyridoxine nutrition in patients with kwashiorkor

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A CHARACTERISTIC clinical syndrome of pyridoxine deficiency has not been described in human adults. Verloop and Rademaker[1] did describe severe hypochromic anemia and hemochromatosis in a 50-year-old man who had subsisted for a period of approximately 3 years in a forced-labor camp on a diet of sour bread, potatoes, and cabbage soup. The anemia remained refractory to all other treatment, but responded to pyridoxine administration. Foy and Kondi[2] also described 2 patients with hypochromic anemia whose condition deteriorated on iron therapy alone; one of these patients responded to pyridoxine treatment. However, none of these 3 cases had clinical signs and symptoms which could be considered as pathognomonic of pyridoxine deficiency.

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Desoxypyridoxine, a metabolic antagonist of the B_6 group of vitamins (pyridoxine, pyridoxamine, and pyridoxal), has been used in experimental studies of vitamin B_6 deficiency in human beings. Vilter and co-workers[3] induced signs and symptoms which they ascribed to vitamin B_6 deficiency in 34 of 50 adult patients who received desoxypyridoxine for 3 to 22 weeks. The main signs and symptoms were anorexia, nausea, lethargy, seborrhea sicca-like dermatitis, cheilosis, glossitis, pellagra-like dermatitis, and polyneuritis. These authors concluded that "vitamin B_6 deficiency mimics the other B complex deficiency diseases."

In 1950 Snyderman, Carretero, and Holt[4] gave a pyridoxine-deficient diet for therapeutic reasons to 2 hydrocephalic infants for 76 and 130 days, respectively. One of the infants developed convulsions which were promptly relieved by the administration of pyridoxine. A severe microcytic hypochromic anemia developed in the other

subject after 130 days. This anemia responded dramatically to pyridoxine; a rise in the reticulocytes was noted after 72 hours. Between 1951 and 1954 several authors,[5, 6] described convulsions in infants who received a commercial liquid-milk formula which contained only about 60 μg of vitamin B_6 per liter. The convulsions were relieved and the electroencephalographic abnormalities found in these infants were corrected by the parenteral administration of vitamin B_6. No other "characteristic" signs and symptoms were noted in these infants. In this connection it is interesting to note that Karlin[7] has suggested that breast-fed infants may have a subclinical deficiency of this vitamin, since the vitamin B_6 content of human milk is relatively low. Human milk may contain as little as 1 to 2 μg per 100 ml. during the first few postpartum days. This author found that the vitamin B_6 content of the milk could be increased by the oral administration of pyridoxine to the mother.

It is obvious that neither in human adults nor in infants has any typical clinical picture of vitamin B_6 deficiency been described. Vilter and colleagues[3] concluded that "in areas where mixed vitamin B complex deficiency states are endemic, the extent of pyridoxine deficiency can be established only by chemical tests." The following are the main biochemical tests which have been suggested as criteria for the assessment of pyridoxine deficiency in the human being.

BIOCHEMICAL TESTS

The excretion of xanthurenic acid in the urine after the administration of a test load of DL-tryptophan.[8, 9] Vitamin B_6 is required for the normal metabolism of tryptophan to nicotinic acid.[10] A deficiency in the vitamin B_6 complex will alter the intermediary metabolism of tryptophan and lead to the excretion of the abnormal metabolite xanthurenic acid (4,8-dihydroxyquinoline-2-carboxylic acid) in the urine. In adults who had received desoxypyridoxine[11] and in pregnant women[12] the excretion of xanthurenic acid was increased after the administration of a test load of tryptophan. In these studies pyridoxine caused a return of the xanthurenic acid excretion to normal levels. Greenberg and associates[8] concluded that "the excretion of xanthurenic acid following test doses of tryptophan is one of the earliest detectable manifestations of pyridoxine deficiency."

The activity of serum glutamic oxalacetic transaminase (SGOT). Pyridoxal-5-phosphate, the enzymatically active form of the vitamin B_6 group of vitamins serves as a coenzyme for SGOT.[13] The transaminase activity in the tissues of pyridoxine-deficient animals was lower than normal[14] and in rats a severe deficiency of vitamin B_6 could be detected readily by measuring the increase in SGOT activity after administration of the vitamin. Babcock, Brush, and Sostman[15] applied the results of their experiments on rats to human beings and concluded that subclinical deficiency of vitamin B_6 caused reduction in SGOT activity. The activity of this enzyme in the sera of "normal" individuals shows considerable variation, and a single determination will be of no value in the detection of subclinical deficiencies. Babcock, Brush, and Sostman,[15] therefore, devised a clinical test which consisted of measurement of the increase in SGOT activity in an individual patient following administration of vitamin B_6. They concluded that "the response of serum glutamic oxalacetic transaminase to oral administration of vitamin B_6 provides a measure of the state of vitamin B_6 nutrition."

The excretion of 4-pyridoxic acid in the urine. This acid is the main urinary metabolite of vitamin B_6.[16] In the two infants who were studied by Snyderman,[4] and who received a pyridoxine-deficient diet "the first change noted was the prompt disappearance of pyridoxic acid from the urine." After a 50 mg. dose of pyridoxine the excretion of pyridoxic acid increased.

The urinary excretion of N-methylnicotinamide (NMN). This nicotinic acid derivative occurs normally in the urine of healthy infants and adults.[17] After ingestion of DL-tryptophan the 24-hour excretion of NMN was markedly increased.[17] The pyridoxine-

Table I. Serum protein levels and serum amylase activity in 13 patients with kwashiorkor

	On admission to hospital (Mean ± S.D.)	*After 14 days' treatment (Mean ± S.D.)*	*After 28 days' treatment (Mean ± S.D.)*	*After 42 days' treatment (Mean ± S.D.)*
Total serum protein (Gm./100 ml.)	3.96 ± 0.18	6.47 ± 0.19	7.54 ± 0.22	7.32 ± 0.14
Albumin (Gm./100 ml.)	1.55 ± 0.23	2.89 ± 0.22	3.60 ± 0.17	3.76 ± 0.16
Globulin (Gm./100 ml.)	2.41 ± 0.14	3.56 ± 0.15	3.94 ± 0.24	3.56 ± 0.15
α-globulin (Gm./100 ml.)	0.63 ± 0.21	1.21 ± 0.11	1.29 ± 0.25	1.29 ± 0.28
β-globulin (Gm./100 ml.)	0.88 ± 0.12	0.81 ± 0.12	0.93 ± 0.14	0.79 ± 0.19
γ-globulin (Gm./100 ml.)	0.97 ± 0.28	1.54 ± 0.14	1.72 ± 0.19	1.47 ± 0.10
Amylase (Somagyi units)	70.8 ± 38.14	140.6 ± 48.57	149.5 ± 39.88	141.4 ± 28.58

deficient infants studied by Snyderman and co-authors[10] lost the ability to convert tryptophan to NMN, and the administration of 0.19 to 0.26 Gm. per kilogram of L-tryptophan caused no increase in the urinary NMN. These infants showed an excellent clinical response to the administration of pyridoxine, but there was a delay in the reappearance of the ability to convert tryptophan to nicotinic acid. Snyderman and her associates considered this test to be a most sensitive indication of vitamin B_6 deficiency in the infant.

METABOLISM OF VITAMINS

Although numerous investigations have been conducted on the metabolism of a great variety of nutrients in patients with kwashiorkor, scant attention has been given to possible abnormalities in vitamin metabolism in this syndrome. In their monograph, Kwashiorkor in Africa, Brock and Autret[18] pointed out that "the diets of Africans, and especially of African children, are never deficient in only one nutrient. In addition to the very marked deficiency in animal protein, there is a deficiency in vegetable proteins, in minerals, and in vitamins (mainly in riboflavine and niacin and sometimes in vitamin A in areas where there is no palm-oil available). It should be added that these are the only vitamin deficiencies which have been looked for and that an interrelationship exists between certain vitamins and amino-acids. It would therefore be a mistake to conclude at this stage that the syndrome is due only to deficiency of one or more amino-acids." In connection with the possible role of pyridoxine deficiency it is interesting to note the results of Abbassy, Zeitoun, and Aboulwafa.[19] These workers determined the xanthurenic acid excretions in 58 "abnormal" children. Twelve of these children were malnourished. "The average xanthurenic acid excretion was high in all cases, but was more pronounced in cases of kwashiorkor." Seven of these patients received vitamin B_6 parenterally (20 mg. twice daily), and the xanthurenic acid estimations were repeated. "In most cases, this led to the return of xan-

Table II. Hematologic values in 13 patients with kwashiorkor

	On admission to hospital (Mean ± S.D.)	*After 14 days' treatment (Mean ± S.D.)*	*After 28 days' treatment (Mean ± S.D.)*	*After 42 days' treatment (Mean ± S.D.)*
Hemoglobin (Gm./100 ml.)	10.5 ± 1.59	9.1 ± 2.05	10.8 ± 1.09	11.2 ± 0.24
Red cell count (millions/cu.mm.)	3.78 ± 0.23	3.23 ± 0.18	3.79 ± 0.15	4.11 ± 0.16
White cell count (thousands/cu.mm.)	8,030 ± 88.24	9,140 ± 161.10	8,130 ± 112.80	9,160 ± 82.30
Packed cell volume (%)	33.2 ± 6.40	31.3 ± 5.30	35.3 ± 4.20	38.0 ± 4.90
Sedimentation rate (mm./hr.)	13.9 ± 13.08	46.3 ± 19.10	36.8 ± 11.70	31.3 ± 9.10

Table III. Total lymphocyte counts in patients with kwashiorkor

Patient No.	*Total lymphocyte count*			
	On admission to hospital	*After 14 days' treatment*	*After 28 days' treatment*	*After 42 days' treatment*
1	2,184	3,528	2,455	4,025
2	4,150	6,324	8,418	4,020
3	6,612	6,109	5,616	5,264
4	4,408	3,591	2,141	2,530
5	4,032	4,032	2,230	2,968
6	3,450	1,221	1,230	5,103
7	1,845	2,870	3,300	3,948
8	4,000	3,364	3,904	4,794
9	3,216	2,592	5,355	6,860
10	2,475	2,628	2,646	3,488
11	4,902	3,813	4,514	--
12	5,355	5,535	5,400	--
13	3,536	5,712	--	--
Mean	4,089	3,947	3,934	4,300

thurenic acid to its normal level suggesting that the increased amount of xanthurenic acid excretion is due to deficiency of this vitamin." These authors did not apply any of the other biochemical criteria mentioned above.

In view of their results and the fact that little attention has been given to possible vitamin deficiencies in kwashiorkor, it was decided to conduct an extensive investigation of the state of pyridoxine nutrition in kwashiorkor patients.

MATERIALS AND METHODS

In the first experiment 13 patients with kwashiorkor were investigated. The patients exhibited the clinical features regarded by Brock and Autret[18] as characteristic of kwashiorkor.

The patients were first treated with the customary skimmed-milk formulas. After a period of approximately 4 days they received a full ward diet in addition to the skimmed milk. During the first week of hospitalization all patients received 300,000 units of a penicillin V suspension every 6 hours. When indicated, broad-spectrum antibiotics were prescribed. In addition all patients received one teaspoonful daily of a multivitamin mixture which did not contain pyridoxine.

Ten of these patients were investigated for a total period of 42 days. Blood and urine were collected on admission to the hospital and at fortnightly intervals thereafter (i.e., a total of 4 collections from each

Table IV. Urinary excretion of xanthurenic acid before and after administration of 0.5 Gm. per kilogram of DL-tryptophan

Patient No.	*Xanthurenic acid (mg./24 hr.)*							
	Admission to hospital		*After 14 days' treatment*		*After 28 days' treatment*		*After 42 days' treatment*	
	Before tryptophan	*After tryptophan*	*Before tryptophan*	*After tryptophan*	*Before tryptophan*	*After tryptophan*	*Before tryptophan*	*After tryptophan*
1	*0.26**	*30.60**	*2.80**	*39.90**	5.80	21.10	3.02	5.80
2	0.20	2.32	*1.96**	*46.50**	5.36	14.3	2.20	8.90
3	0.79	14.10	3.76	19.60	3.15	30.90	2.44	8.91
4	*5.04**	*53.80**	6.50	22.40	2.60	10.40	2.40	5.90
5	5.31	11.90	4.60	11.50	3.20	20.10	6.10	11.70
6	0.73	15.45	1.20	10.40	2.40	6.60	1.50	1.80
7	*2.30**	*35.60**	2.60	15.10	1.40	14.10	3.40	5.70
8	4.60	6.60	1.20	3.70	24.20	24.40	22.40	22.50
9	1.20	3.60	3.20	9.80	2.90	6.70	2.10	2.30
10	2.60	18.40	3.20	7.20	1.80	2.80	1.10	1.20
11	1.10	5.30	0.00	3.10	1.00	1.20	--	--
12	0.60	16.00	9.80	11.10	1.80	2.00	--	--
13	1.70	6.80	1.60	2.40	--	--	--	--
Mean	2.03	16.96	3.26	15.6	4.49	12.71	4.85	7.66
± S.D.	1.76	14.33	2.47	13.15	5.61	9.04	6.58	6.44

*Patients with a xanthurenic acid excretion above 30 mg. per 24 hours.

patient). At each collection the following procedure was followed:

After approximately 12 ml. of blood had been taken from the external jugular vein, a 24-hour specimen of urine was collected. The patient then received 0.5 gram per kilogram of DL-tryptophan dissolved in skimmed milk by mouth, and a second 24-hour urine specimen was collected.

Immediately after the third series of collections each of these 10 patients received 12.5 mg. of pyridoxine hydrochloride intramuscularly, daily, for a total period of 14 days.

Two patients were investigated for a total period of 28 days (i.e., 3 collections). In one case only 2 collections of blood and urine could be carried out. Apart from the vitamin B_6 provided in the diet, these 3 patients did not receive any additional supplements of pyridoxine.

In the second experiment 5 patients with kwashiorkor were investigated. Blood and urine were collected on admission to the hospital as described above. Immediately after this each patient received daily intramuscular injections of 12.5 mg. of pyridoxine hydrochloride for a total period of 7 days, and blood and urine were again collected.

Biochemical determinations on blood and urine. The following investigations were carried out on the blood specimens: serum glutamic oxalacetic transaminase (SGOT) activity,[20] amylase,[21] and serum protein (total; albumin; globulin, alpha, beta, and gamma globulin).[22] Hemoglobin was determined as oxyhemoglobin according to the method of King and co-authors.[23] Red cell count, white cell count, differential count, packed cell volume (PCV), and erythrocyte sedimentation rate (ESR) were determined according to standard methods.[24]

In the urine, the following determinations were performed: xanthurenic acid (XA),[12] 4-pyridoxic acid,[25] and N-methylnicotinamide (NMN).[26]

RESULTS

The results of the serum protein and serum amylase determinations on the 13

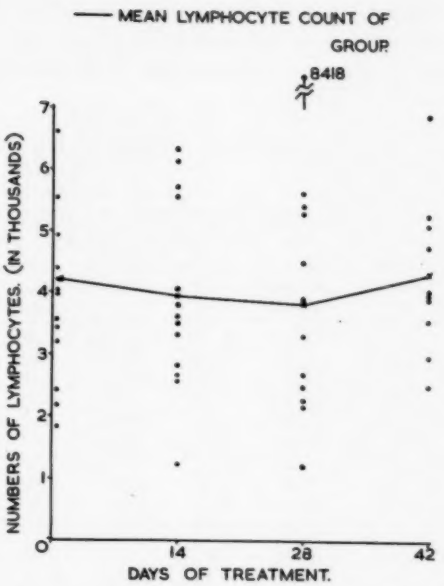

Fig. 1. Total lymphocyte counts in patients with kwashiorkor.

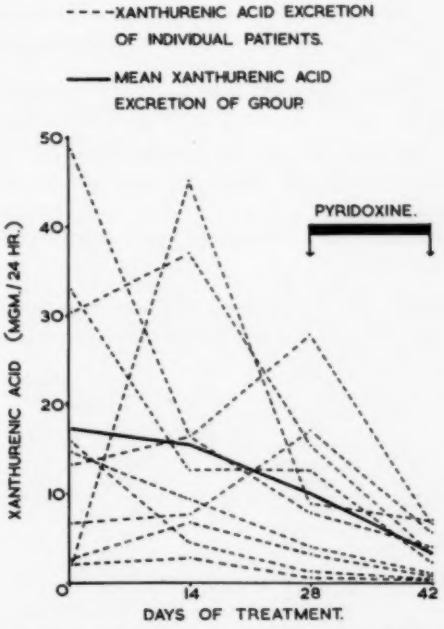

Fig. 2. Xanthurenic acid excretion in the urine of 10 patients with kwashiorkor.

Table V. Increase in xanthurenic acid excretion after administration of 0.5 Gm. per kilogram of DL-tryptophan in 10 patients with kwashiorkor

	Xanthurenic acid (mg./24 hr.)			
	Before pyridoxine			*After pyridoxine*
Patient No.	*Admission to hospital*	*After 14 days' treatment*	*After 28 days' treatment*	*After 42 days' treatment*
1	*30.34**	*37.10**	15.30	2.78
2	2.12	*44.54**	8.94	6.70
3	13.31	15.84	27.75	6.47
4	*48.76**	15.90	7.80	3.50
5	6.59	6.90	10.90	5.60
6	14.72	9.20	4.20	0.30
7	*33.30**	12.50	12.70	2.30
8	2.00	2.50	0.20	0.10
9	2.40	6.60	3.80	0.20
10	15.80	4.00	1.00	0.10
Mean	16.93	15.51	9.86	2.80
+ S.D.	14.97	13.46	8.08	2.55

*Patients with a xanthurenic acid excretion above 30 mg. per 24 hours.

patients in the first experiment are presented in Table I.

These results are in accordance with those reported by other workers in kwashiorkor cases.[27]

The hematologic data are listed in Table II.

Anemia was present in the majority of patients, but, as found in other studies of kwashiorkor, the severity and morphologic characteristics were extremely variable.[27] The unusual changes in the sedimentation rate which occurred in these patients have been discussed in a previous publication.[28]

During their investigations on pyridoxine deficiency induced by desoxypyridoxine, Vilter and his associates[3] noted that 17 of the 18 patients developed an absolute lymphocytopenia. After administration of pyridoxine the total lymphocyte counts increased to pretreatment levels. The total lymphocyte counts in our patients with kwashiorkor are shown in Table III and Fig. 1.

It is obvious from this table and from Fig. 1 that there was no general tendency toward an absolute lymphocytopenia in the group as a whole. It is, however, interesting to note that in 8 of the 10 patients who received pyridoxine between the twenty-eighth and forty-second days of treatment, the total lymphocyte count increased.

Most of the patients had signs and symptoms of infections, including bronchopneumonia and primary pulmonary tuberculosis, which may have caused a slight lymphocytosis in some cases. It is possible that this lymphocytosis prevented the occurrence of an absolute lymphocytopenia which is a frequent finding in pyridoxine-deficient patients according to Vilter.[3]

Urinary excretion of xanthurenic acid. The amounts of xanthurenic acid excreted in the urine before and after administration of 0.5 Gm. per kilogram of DL-tryptophan are found in Table IV.

Table VI. Xanthurenic acid excretion before and after administration of 0.5 Gm. per kilogram of DL-tryptophan in 5 patients with kwashiorkor

	Xanthurenic acid (mg./24 hr.)					
	Before pyridoxine			*After pyridoxine*		
	On admission to hospital			*After 7 days' treatment*		
Patient No.	*Before tryptophan*	*After tryptophan*	*Increase**	*Before tryptophan*	*After tryptophan*	*Increase**
14	1.45	4.04	2.59	0.10	0.67	0.57
15	0.10	5.04	4.94	3.96	6.80	2.84
16	0.10	3.33	3.23	1.43	1.65	0.22
17	1.23	14.30	13.07	1.05	4.26	3.21
18	0.10	5.12	5.02	0.55	2.34	1.79
Mean	0.60	6.37	5.77	1.42	3.14	1.73
± S.D.	0.61	4.02	3.77	1.35	2.17	1.19

*Increase in xanthurenic acid excretion after administration of tryptophan.

Table VII. Urinary excretion of 4-pyridoxic acid in 13 patients with kwashiorkor

Patient No.	4-pyridoxic acid (mg./24 hr.)							
	Before pyridoxine						After pyridoxine	
	Admission to hospital		After 14 days' treatment		After 28 days' treatment		After 42 days' treatment	
	Before tryptophan	After tryptophan	Before tryptophan	After tryptophan	Before tryptophan	After tryptophan	Before tryptophan	After tryptophan
1	--	--	< 0.10*	0.20	0.27	0.54	3.0	4.4
2	--	--	0.11	0.52	3.20	4.40	7.90	> 8.00*
3	< 0.10*	< 0.10	0.45	0.33	0.47	0.20	> 8.00	> 8.00
4	< 0.10	0.21	0.31	0.39	1.10	0.60	> 8.00	> 8.00
5	< 0.10	< 0.10	0.34	0.41	0.47	0.48	3.90	6.40
6	< 0.10	< 0.10	0.12	0.14	0.28	0.10	> 8.00	> 8.00
7	< 0.10	0.21	0.12	0.30	0.44	0.29	> 8.00	> 8.00
8	0.17	0.26	0.25	0.17	0.26	0.31	> 8.00	> 8.00
9	0.11	0.10	0.11	0.14	0.95	1.20	> 8.00	> 8.00
10	< 0.10	< 0.10	0.14	0.49	0.85	1.50	> 8.00	> 8.00
11	< 0.10	< 0.10	1.30	1.30	2.40	2.60	--	--
12	0.19	0.15	1.70	1.60	1.80	1.90	--	--
13	< 0.10	0.13	0.36	0.41	--	--	--	--

*Because of the unreliability of the method when either very small or very large amounts of 4-pyridoxic acid are excreted, amounts lower than 0.1 mg. per 24 hours are given as < 0.10, and higher than 8.0 mg. per 24 hours as > 8.00.

In all cases the excretion of xanthurenic acid increased after administration of DL-tryptophan. The mean increase in xanthurenic acid excretion of the group as a whole amounted to 14.93 mg. per 24 hours on admission to the hospital, 12.34 mg. after 14 days of treatment, 8.22 mg. after 28 days of treatment, and 2.81 mg. after 42 days of treatment. In 4 individual patients (Nos. 1, 2, 4, and 7, Table IV) the amount of xanthurenic acid excreted in the urine during 24 hours increased to levels higher than 30 mg.

If the increase in xanthurenic acid excretion after administration of tryptophan found in the 10 patients who received 12.5 mg. pyridoxine intramuscularly daily from the twenty-eighth day of treatment is considered (Table V and Fig. 2), it will be noted that the excretion of xanthurenic acid decreased in all 10 patients after administration of pyridoxine.

Although the mean xanthurenic acid excretion of the group (Fig. 2 and Table V) showed a steady decline during the first 28 days of treatment (i.e., during the period when the patients received either skimmed milk or skimmed milk plus ward diet), there was considerable variation in the pattern of xanthurenic acid excretion among individual patients. The only constant feature was the fact that after 28 days of treatment when the patients received extra pyridoxine intramuscularly, the xanthurenic acid excretion

Table VIII. Serum glutamic oxalacetic transaminase (SGOT) activity in patients with kwashiorkor

Patient No.	SGOT activity (Caboud units)			
	Before pyridoxine			After pyridoxine
	Admission to hospital	After 14 days' treatment	After 28 days' treatment	After 42 days' treatment
1	17.0	12.0	14.0	10.5
2	11.5	15.0	12.5	10.5
3	18.0	19.0	14.0	14.5
4	10.0	11.0	9.0	13.5
5	8.0	10.0	14.5	20.0
6	22.0	21.0	13.0	31.0
7	17.0	11.0	10.0	9.5
8	20.0	20.0	12.0	4.0
9	21.0	14.0	14.0	19.0
10	18.0	5.0	10.5	12.0
11	63.0	22.0	11.0	--
12	9.5	10.0	17.0	--
13	17.0	6.5	--	--
Mean ± S.D.	19.4 ±13.34	13.6 ± 5.31	12.6 ± 2.19	14.5 ± 7.05

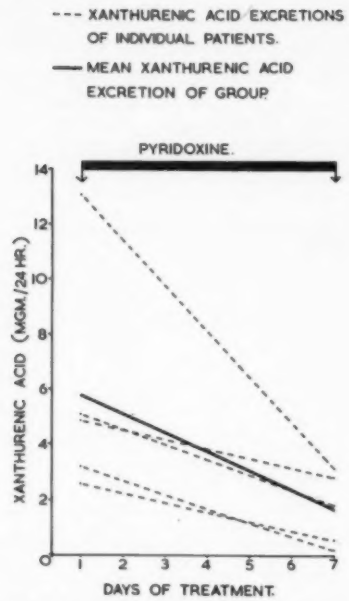
--- XANTHURENIC ACID EXCRETIONS OF INDIVIDUAL PATIENTS.
— MEAN XANTHURENIC ACID EXCRETION OF GROUP
PYRIDOXINE.
XANTHURENIC ACID (MGM./24 HR.)
DAYS OF TREATMENT.

Fig. 3. Xanthurenic acid excretions in the urine of patients with kwashiorkor during the first 7 days of treatment.

decreased in all cases (Fig. 2). This decrease was most marked in the patients who excreated relatively large amounts of xanthurenic acid on the twenty-eighth day.

The xanthurenic acid excretions of the 5 patients in the second experiment are presented in Table VI.

The results in Table VI show that there was an increase in xanthurenic acid excretion in all the patients after administration of tryptophan. The level of xanthurenic acid excretion decreased in all cases after pyridoxine administration (Fig. 3).

Urinary excretion of 4-pyridoxic acid. The amounts of 4-pyridoxic acid excreted in the urine of the 13 patients in the first experiment are presented in Table VII.

These results (Table VII) indicate that there was a variable response to administration of tryptophan among individual patients; in some cases the amount of 4-pyridoxic acid excreted in the urine increased after tryptophan loading, while in others a decrease occurred. It is clearly evident from the results presented in Table VII that the excretion of 4-pyridoxic acid was extremely low on admission to the hospital (< 0.10 to 0.26 mg. per 24 hours). In all patients the urinary excretion of this metabolite increased steadily during the course of treatment. After administration of pyridoxine a marked increase occurred. These results are presented graphically in Fig. 4. In this figure only the "basal" amounts of 4-pyridoxic acid excreted by individual patients at each collection are illustrated, i.e., the amounts excreated before administration of tryptophan.

Serum glutamic oxalacetic transaminase (SGOT) activity. The activity of this enzyme was determined in the sera of the 13 patients in the first experiment. These results are shown in Table VIII.

Although the SGOT activity of certain individual patients increased after pyridoxine administration (e.g., Nos. 5 and 6, Table VIII), the activity of this enzyme decreased in other patients. It is obvious that the response to vitamin B_6 administration was extremely variable.

N-methylnicotinamide (NMN) excretion in the urine. The urinary excretion of N-methylnicotinamide was determined on ad-

Table IX. Urinary excretion of N-methylnicotinamide (NMN) before and after administration of DL-tryptophan

Patient No.	*NMN (mg./24 hr.)*		*Increment index**
	Before tryptophan	*After tryptophan*	
1	0.77	2.20	2.8
2	5.90	10.00	1.7
3	1.50	13.10	8.7
4	3.40	13.70	4.0
5	3.30	3.60	1.1
6	2.00	7.50	3.7
7	4.80	14.80	3.1
8	5.90	14.70	2.4
9	1.31	2.31	1.8
10	4.60	6.80	1.5
11	6.90	7.20	1.0
12	3.40	4.50	1.3
Mean	3.65	8.37	2.77
± S.D.	1.92	4.58	2.03

*Increment index: $\frac{\text{NMN after tryptophan}}{\text{NMN before tryptophan}}$.

Table X. Urinary excretion of N-methylnicotinamide (NMN) before and after administration of tryptophan

Patient No.	NMN (mg./24 hr.)					
	Admission to hospital			After 7 days' treatment		
	Before tryptophan	After tryptophan	Increment index	Before tryptophan	After tryptophan	Increment index
14	11.24	6.71	−1.7	3.16	4.05	1.3
15	1.22	2.41	1.9	5.21	4.45	−1.2
16	0.79	3.40	4.3	0.99	15.10	15.2
17	1.46	2.83	1.9	3.48	13.11	3.8
18	2.44	1.54	−1.6	4.44	11.41	2.0
Mean	3.43	3.38	0.96	3.42	9.62	4.22
± S.D.	3.94	1.77	2.30	1.51	4.54	5.72

mission to the hospital in 12 of the 13 patients in the first experiment. The results are listed in Table IX.

These results show that the excretion of NMN in the urine increased 1.0 to 8.7 fold in these 12 patients after administration of tryptophan. In 2 of the patients (Nos. 5 and 11) the increment index was less than 1.2.

The urinary excretion of NMN was also determined in the 5 patients in the second experiment on admission to the hospital and after one week of treatment (Table X).

In 3 of these patients (Nos. 14, 15, and 18) a negative increment index was found. Two of these patients showed a negative index on admission to the hospital, while the third patient (No. 15) lost the ability to convert tryptophan to NMN after one week of treatment in the hospital. Except in the case of this patient the increment index gave higher values after one week of treatment than on admission to the hospital.

DISCUSSION

Several of the biochemical criteria used in this investigation indicated a need for vitamin B_6 in patients with kwashiorkor.

The results of the xanthurenic acid determinations showed that the excretion of this abnormal metabolite increased after administration of 0.5 Gm. per kilogram of DL-tryptophan. Rabe and Plonko[29] studied patients with anemia, convulsions, and acute infections and concluded that "the appearance of xanthurenic acid in the urine of children less than 4 years of age following a DL-tryptophan load of 0.54 Gm. per kilogram indicates a need for pyridoxine." In 4 of the patients with kwashiorkor investigated by us, the excretion of xanthurenic acid reached levels which were higher than 30 mg. per 24 hours. According to Knapp and Gassmann[30] this must be considered as definite evidence of pyridoxine deficiency. Furthermore, in all the patients who received pyridoxine hydrochloride parenterally (12.5 mg. daily for periods of 7 to 14 days) the amount of xanthurenic acid excreted in the urine decreased.

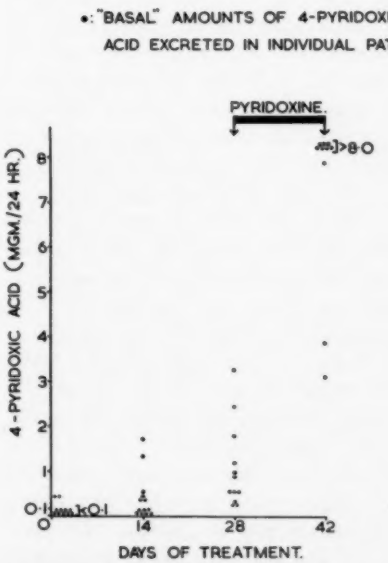

Fig. 4. Urinary excretion of 4-pyridoxic acid.

In all the patients the excretion of 4-pyridoxic acid, the main urinary metabolite of vitamin B_6, was extremely low on admission to the hospital (< 0.1 to 0.26 mg. per 24 hours). In all patients the excretion of this metabolite increased steadily during treatment with the customary skimmed-milk formulas and ward diet. After parenteral administration of pyridoxine a marked increase occurred and in 7 of 13 patients the amounts of 4-pyridoxic acid excreted were higher than 8.0 mg. per 24 hours. In the investigations of Snyderman and associates[10] no 4-pyridoxic acid could be determined in the urine after cessation of pyridoxine ingestion in one child. In the second baby this acid was still excreted in small amounts up to 40 days after complete withdrawal of pyridoxine. These workers and Rabe and Plonko[29] noted an increased excretion of 4-pyridoxic acid after parenteral administration of pyridoxine. It is, therefore, probable that the excretion of extremely small amounts of 4-pyridoxic acid (< 0.1 mg. per 24 hours) followed by an increased excretion after administration of vitamin B_6 can be considered as evidence of pyridoxine need.

Perlzweig and co-workers[17] found increased amounts of N-methylnicotinamide in the urine of apparently normal infants and adults after ingestion of tryptophan. The excretion of NMN increased to levels which were 2 to 6 times higher than the amounts excreted before administration of the amino acid. On the basis of these results and the findings of Snyderman and associates,[10] Rabe and Plonko[29] concluded that urinary NMN increments of 1.2 or greater after a standard tryptophan load were normal and that values less than this indicated a pyridoxine need. In our investigation 5 out of 17 kwashiorkor patients had a NMN "increment index" lower than 1.2. In 3 of these patients a negative "index" was found.

In our investigation the 3 biochemical criteria discussed above, viz., xanthurenic acid and NMN excretion before and after a tryptophan load and 4-pyridoxic acid excretion before and after pyridoxine administration, all indicated a need for pyridoxine in the group of patients with kwashiorkor. It must be pointed out, however, that there was no consistent evidence for pyridoxine need in individual patients when the results of these 3 tests were compared. Patient No. 4, for instance, had a xanthurenic acid excretion which was definitely abnormal on admission to the hospital (48.76 mg. per 24 hours after tryptophan load). The amount of 4-pyridoxic acid excreted by this patient was also extremely low (< 0.10 mg. per 24 hours), but the NMN "increment index" was 4.0, which is within the normal range. This discrepancy has been noted by other workers.[29, 31] The latter workers concluded that this discrepancy was due "possibly to the fact that each of the tests varies in its sensitivity in detecting pyridoxine need." In the present investigation there was fairly close agreement between the results of the xanthurenic acid and 4-pyridoxic acid determinations. Since the determination of xanthurenic acid is technically simpler than that of 4-pyridoxic acid, it is suggested that estimation of this abnormal metabolite before and after a tryptophan load should be used in future studies of pyridoxine need in patients with kwashiorkor.

The other biochemical criteria which were applied, viz., total lymphocyte counts and SGOT activity, gave no consistent evidence of pyridoxine need in the group of kwashiorkor cases. It is probable that these criteria are affected by factors other than the state of pyridoxine nutrition, e.g., secondary infections and functional disorders of the liver.

Finally, it must be emphasized that the patients who showed evidence of pyridoxine need according to one or more of the biochemical criteria described above had no distinctive clinical features other than those usually encountered in patients with kwashiorkor.

SUMMARY

Various biochemical criteria were applied in a study of the state of pyridoxine nutrition in 18 patients with kwashiorkor. There was evidence of pyridoxine need in 4 patients who excreted large amounts of xan-

thurenic acid in the urine after a tryptophan load, and in 5 other patients who lost the ability to convert tryptophan to niacin. In all patients the amount of xanthurenic acid excreted in the urine decreased after parenteral administration of pyridoxine hydrochloride. The excretion of 4-pyridoxic acid was extremely low in all patients on admission to the hospital but increased gradually during dietary treatment, while a marked increase occurred after administration of pyridoxine hydrochloride.

The other criteria used in this investigation (viz., total lymphocyte counts, glutamic oxalacetic transaminase activity of the serum) revealed no definite evidence of pyridoxine need.

It is suggested that determination of urinary xanthurenic acid excretion, before and after a tryptophan load, should be used in future studies of the state of pyridoxine nutrition in patients with kwashiorkor.

REFERENCES

1. Verloop, M. C., and Rademaker, W.: Anaemia Due to Pyridoxine Deficiency in Man, Brit. J. Haemat. **6:** 66, 1960.
2. Foy, H., and Kondi, A.: Hypochromic Anemias of the Tropics Associated With Pyridoxine and Nicotinic Acid Deficiency, Blood **13:** 1054, 1958.
3. Vilter, R. W., Mueller, J. F., Glazer, H. S., Jarrold, T., Abraham, J., Thompson, C., and Hawkins, V. R.: The Effect of Vitamin B_6 Deficiency Induced by Desoxypyridoxine in Human Beings, J. Lab. & Clin. Med. **42:** 335, 1953.
4. Snyderman, S. E., Carretero, R., and Holt, L. E.: Pyridoxine Deficiency in the Human Being, Fed. Proc. **9:** 371, 1950.
5. Coursin, D. B.: Convulsive Seizures in Infants With Pyridoxine Deficient Diet, J. A. M. A. **154:** 406, 1954.
6. Moloney, C. J., and Parmelee, A. H.: Convulsions in Young Infants as a Result of Pyridoxine (Vitamin B_6) Deficiency, J. A. M. A. **154:** 405, 1954.
7. Karlin, R.: in Vitamin B_6 in Human Milk, Nutrition rev. **18:** 136, 1960.
8. Greenberg, L. D., Bohr, D. F., McGrath, H., and Rinehart, J. F.: Xanthurenic Acid Excretion in the Human Subject on a Pyridoxine Deficient Diet, Arch. Biochem. **21:** 237, 1949.
9. Wachstein, M., and Gudaitis, A.: Detection of Vitamin B_6 Deficiency: Utilization of Improved Method for Rapid Determination of Xanthurenic Acid in Urine, Am. J. Clin. Path. **22:** 652, 1952.
10. Snyderman, S. E., Holt, L. E., Carretero, R., and Jacobs, K.: Pyridoxine Deficiency in the Human Infant, J. Clin. Nutrition **1:** 200, 1953.
11. Glazer, H. S., Mueller, J. F., Thompson, C., Hawkins, V. R., and Vilter, R. W.: A Study of Urinary Excretion of Xanthurenic Acid and Other Tryptophane Metabolites in Human Beings With Pyridoxine Deficiency Induced by Desoxypyridoxine, Arch. Biochem. **33:** 243, 1951.
12. Wachstein, M., and Gudaitis, A.: Disturbance of Vitamin B_6 Metabolism in Pregnancy, J. Lab. & Clin. Med. **40:** 550, 1952.
13. Krehl, W. A.: Vitamin B_6 in Nutrition and Metabolism, Borden Rev. Nutrition Res. **18:** 69, 1957.
14. Schlenk, F., and Snell, E. E.: Vitamin B_6 and Transamination, J. Biol. Chem. **157:** 425, 1945.
15. Babcock, M. J., Brush, M., and Sostman, E.: Evaluation of Vitamin B_6 Nutrition, J. Nutrition **70:** 369, 1960.
16. Sarett, H. P.: A Study of the Measurement of 4-Pyridoxic Acid in Urine, J. Biol. Chem. **189:** 769, 1951.
17. Perlzweig, W. A., Rosen, F., Levitas, N., and Robinson, J.: The Excretion of Nicotinic Acid Derivatives After Ingestion of Tryptophane by Man, J. Biol. Chem. **167:** 511, 1947.
18. Brock, J. F., and Autret, M.: Kwashiorkor in Africa, WHO Monograph No. 8: 54, 1952.
19. Abbassy, A. S., Zeitoun, M. M., and Aboulwafa, M. H.: The State of Vitamin B_6 Deficiency as Measured by Urinary Xanthurenic Acid, J. Trop. Pediat. **5:** 45, 1959.
20. Mohun, A. F., and Cook, I. J. Y.: Simple Methods for Measuring Serum Levels of the Glutamic-Oxalacetic and Glutamic-Pyruvic Transaminases in Routine Laboratories, J. Clin. Path. **6:** 394, 1957.
21. Smith, B. W., and Roe, J. H.: A Photometric Method for the Determination of α-Amylase in Blood and Urine, With Use of the Starch-Iodine Color, J. Biol. Chem. **179:** 53, 1949.
22. Robinson, H. W., and Hogden, C. G.: The Biuret Reaction in the Determination of Serum Proteins. II. Measurements Made by a Duboscq Colorimeter Compared With Values Obtained by the Kjeldahl Procedure, J. Biol. Chem. **135:** 727, 1940.
23. King, E. J., Bartholomew, K. J., Geiser, M., Ventura, S., and Wootton, J. D.: Determination of Haemoglobin, Lancet **1:** 1044, 1951.
24. Wintrobe, M. M.: Clinical Hematology, ed. 3, London, 1951, Henry Kimpton.
25. Reddy, S. K., Reynolds, M. S., and Price, J. M.: The Determination of 4-Pyridoxic Acid in Human Urine, J. Biol. Chem. **233:** 691, 1958.
26. Karpenter, K. J., and Kodicek, E.: Fluorimetric Estimation of N-Methylnicotinamide, Biochem. J. **46:** 421, 1950.
27. Gomez, F., Gawan, R. R., Gravioto, J., and

Frenk, S.: Advances in Pediatrics, vol. 7, Chicago, 1955, Year Book Publishers, Inc.
28. Catzel, P., Theron, J. J., and Pretorius, P. J.: The Erythrocyte Sedimentation Rate in Kwashiorkor (With an Analysis of Some of the Biochemical and Haematological Factors Concerned), J. Trop. Pediat. **4:** 98, 1958.
29. Rabe, E. F., and Plonko, M.: Pyridoxine Hydrochloride (Vitamin B_6) Need in Infants and Children, A. M. A. J. Dis. Child. **92:** 382, 1956.
30. Knapp, A., and Gassman, B.: Ueber eine neue Hereditäre von vitamin B_6-abhängige Störung im Tryptophan-Stoffwechsel, Clin. chim. acta **5:** 6, 1960.
31. Cheslock, K. E., and McCully, M. T.: Response of Human Beings to a Low-Vitamin B_6 Diet, J. Nutrition **70:** 507, 1960.

CLINICAL PATHOLOGICAL CONFERENCE

THE CHILDREN'S HOSPITAL MEDICAL CENTER

BOSTON, MASS.

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THIS 6-week-old girl was admitted from the Medical Outpatient Department of The Children's Hospital Medical Center. The family history indicates that a sibling ($2^{10}/_{12}$ years) has cystic fibrosis, whereas the other sibling ($1^{10}/_{12}$ years) is normal. The father is a tree specialist. The family lives in a trailer.

The child was born at term weighing 6 pounds, 6 ounces, after an uneventful pregnancy and a difficult 1½ hours of labor. At 2 days of age the mother noted a scaly condition of the skin of the abdomen and chest. This increased despite use of baby oil. At 7 days of age, there were scaly areas over the face and arms, spreading all over the body in the next 2 days. No red areas or sores were found. The condition persisted until admission, although the baby was bathed daily with Ivory soap and water and anointed with baby oil and petroleum jelly afterward. She was fed, daily, an evaporated milk formula 3 times and 10 drops of multivitamins. At 2 weeks of age, 1 tablespoon of Pablum was added twice daily. The infant cried and sucked the fingers a great deal and was thought to have regurgitated three fourths of the feeding over the past week or so. For the week preceding admission, she had 3 to 4 loose watery stools daily and had become swollen in the feet, hands, and jaw. She entered another hospital for 3 days and was referred here for study.

Examination revealed a temperature of 96.6° F.; pulse 140 per minute; respirations 30 per minute; weight 6 pounds, 8 ounces; and a dark-skinned, scarcely moving infant (Fig. 1). There was extensive scaling of the thin parchmentlike shiny skin over the whole body, leaving fresh normal-appearing skin beneath except for linear erosions in the creases about the groins and genitals. Apart from 2 plus pitting edema of the feet and legs, a thick white vaginal discharge, and a slight nasal drainage, there were no other abnormalities in the entire examination. Treatment of the skin consisted of sulfur-salicylic acid ointment to the scaling areas on the scalp followed with phisoHex shampoo and Bacitracin ointment over the remainder of the body. The intertriginous areas were successfully treated for monilial involvement. Despite transfusion of whole blood, the edema remained severe enough to

Fig. 1. Edema and exfoliation are evident in this dark-skinned child.

close the eyes and apparently to interfere with sucking. On the third hospital day, exertional circumoral cyanosis and rapid shallow respirations with clear breath sounds were followed with an episode of aspiration. The patient was treated with chloramphenicol and penicillin intravenously. Respirations became labored, urine output decreased, and edema increased. Right middle lobe atelectasis and irregular aeration were demonstrated on the fifth hospital day. On this day, an erythematous maculopapular rash involved face and back, and there were small vesicles on the feet. Viokase and Nutramigen were begun. On the average daily intake of 10 ounces, a 6 ounce weight gain between the fourth and fifth days was followed by a similar weight loss after administration of albumin intravenously. After 3 infusions totaling 15 Gm. of albumin, the serum albumin rose from 2.9 to 3.2 Gm. per cent. Left upper lobe atelectasis was demonstrated after an episode of apnea on the seventh day. The improved skin lesions showed more crusting on the face. Rhonchi were constantly present in the chest. The child was distressed and cyanotic when lying on the right side. Erythromycin and chloromycetin were given. The patient died on the ninth hospital day.

The urine was cloudy, the pH was 5.5. It contained albumin, 0; sugar, 1 plus; acetone, 0; bile, 0; red blood cells, 6 to 8; white blood cells, 8 to 10; and many bacteria. The hemoglobin was 6.7 Gm. per cent; hematocrit, 22 per cent; red blood cells, 2.31 million per cubic millimeter; white blood cells, 37,800 per cubic millimeter with 86 per cent polymorphonucelar leukocytes, 11 per cent lymphocytes, and 3 per cent monocytes. Red cells showed hypochromia, anisocytosis, poikilocytosis, and the reticulocyte count was 4.2 per cent. Lumbar puncture fluid was normal. The nonprotein nitrogen was 14.5 mg. per cent. The albumin-globulin ratio was 2.9 to 0.7 Gm. per cent.

Electrophoresis showed albumin, 36 per cent; globulin, alpha$_1$, 12 per cent; alpha$_2$, 20 per cent; beta, 11 per cent; and gamma, 21 per cent.

The pH of the serum was 7.45; carbon dioxide, 25.2 mEq. per liter; sodium, 138 mEq. per liter; potassium, 6.2 mEq. per liter; chloride, 106 mEq. per liter; bilirubin, 0.2 mg. per cent direct and 1.0 mg. per cent total; cholesterol, 95 mg. per cent; protein-bound iodine, 2.6 μg per cent, vitamin A, 9 μg per cent; and serum carotene, 10 μg per cent. A blood culture gave no growth. The Hinton test was negative. A sweat plate was negative 3 times. Iontophoresis sweat test gave quantities insufficient for study. Stool trypsin was 4 plus on admission; negative 3 times, thereafter. No pathogens grew in stool culture. Nose, throat, and scalp culture grew coagulase positive *Staphylococcus aureus,* phage types 55 and 39, and *Bacillus proteus.*

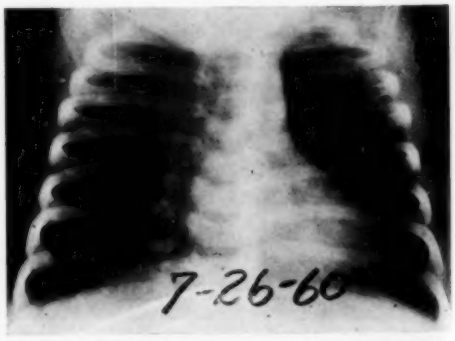

Fig. 2. Slightly irregular hyperaeration is evident.

DR. GORDON F. VAWTER. This photograph of the patient on the second hospital day (Fig. 1) shows both the marked generalized edema and the extensive scaling process involving skin. The pigmentation may well be related to the fact that the child's parents are of Portuguese extraction.

DR. ROBERT D. GRIESEMER.* I had the pleasure of seeing this child in the hospital, but I don't know what the pathologists found. I was somewhat confused by what I saw then.

First, I would like to highlight some aspects of the case. There was a family history of cystic fibrosis of the pancreas. Next, this rash began shortly after birth and reached maximum by the age of 9 days. It began on the surface of the abdomen and then spread to the face, arms, and other areas. Apparently there was never any erythema or blistering. This was an exfoliative process from the start, apparently lasting 5 weeks, and was still present when the patient was admitted to the hospital. During the hospital stay the striking thing was that the scaling did seem to improve and before death I think the skin was nearly clear. I am not sure that this was due to the therapy that I prescribed. It may have been the natural course of the illness. We used only a mild peeling agent, sulfur-salicylic acid ointment. One of the interesting features was the tendency to fissuring, with large cracks between flat scaling areas over the face and body at the height of the exfoliative process. There were many defects in the skin, which could serve as portals of entry for various things.

Other symptoms appeared at 5 weeks of age: regurgitation of food and watery bowel movements. At about the same time edema made its appearance on the hands, feet, and face. There may have been some urinary infection. There was marked anemia and also a polymorphonuclear leukocytosis. The albumin and globulin fractions were quite

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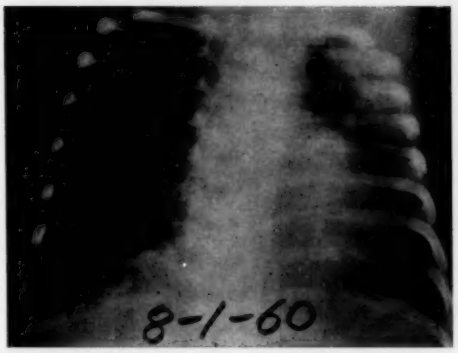

Fig. 3. Atelectasis of right middle lobe and hazy densities are seen.

low. Later the albumin seemed to rise, probably as a result of intravenous injection of albumin. The Hinton test was negative. Of great interest, of course, is the fact that the sweat test for cystic fibrosis was negative, but she could have had cystic fibrosis which was not detected by Dr. Shwachman's very elegant test, because the exfoliative dermatitis might have inhibited sweating.

I would like to discuss this case starting with the cause of death. Apparently, death was partly from respiratory causes; quite probably atelectasis and infection were present in the lungs. I cannot be sure whether pulmonary edema developed following the first injection of serum albumin, with its accompanying increase in body weight. What role such pulmonary edema may have had in the death, 4 days later, would remain problematic, too. However, I have seen an adult with marked exfoliative dermatitis who developed hypoproteinemia and then edema, in whom administration of 50 Gm. of albumin was followed by fatal pulmonary edema.

Hypoproteinemia looms large in this infant. Now, where did this come from? Was it a result of protein loss by exfoliation of skin? I am not sure the process of exfoliation was severe enough for that to be the only mechanism. Could the serum have leaked through the large cracks—the "fissures" that I mentioned? This is a possibility. Adults

who exfoliate with leakage of fluid through the skin are the patients who develop hypoproteinemia most rapidly, as one would expect. In addition, such adults with exfoliative dermatitis have changes in the liver which may affect the rate of albumin synthesis. We have no definitive information on this in the present case; perhaps the pathologist can help us. I doubt that poor nutrition accounts for all of this, but we must consider malabsorption on the basis of pancreatic insufficiency. There are thus many potential causes of hypoproteinemia here, but I am not sure what importance to assign to any particular one.

We have an anemia which may have resulted from the inanition or from infection and I think that at this point I would like Dr. Shahidi's opinion on the blood findings.

DR. NASROLLAH SHAHIDI. The protocol describes hypochromia but calculating the indices suggests neither microcytosis nor hypochromia; this obviously rules out the possibility of iron deficiency anemia. As you pointed out, I think that this anemia is probably the result of protein failure, and we have had similar cases previously. Decreased hemoglobin synthesis under such circumstances has been worked out pretty well in animals. In humans the best example is illustrated in patients with kwashiorkor. The patient with kwashiorkor, however, may suffer from vitamin deficiency too, and this could be masked. Two years ago we studied 2 cases very similar to the one described here. When we treated these 2 infants with protein, without any iron or other hematopoietic agents like vitamin B_{12} and folic acid, there was a good reticulocytosis and the hemoglobin came up very nicely. We thought that this infant (discussed today) was too ill to stand such a regimen.

DR. GRIESEMER. Did you think that the white count indicated infection?

DR. SHAHIDI. I would think so.

DR. GRIESEMER. Bacteria of low virulence, always present on the skin, could penetrate through the cracks in this child's skin and possibly give rise to the picture in the blood count. In the adult with exfoliative dermatitis, the mild infection that is set up in the skin leads to very pronounced lymphadenopathy and one finds an enormous increase in the size of the lymph nodes to golf-ball size, all over the body. At times these patients have been thought to have lymphoma, but histologic examination of nodes shows very clearly that this is not the case.

Let us now consider the early stage of scaling, before all these complications occurred—scaling that was present at birth, and possibly in utero. Is this a primary or a secondary process? Scaling, in general, is the end stage of inflammation. There are, then, many causes for scaling. The most familiar to all of us is sunburn with its subsequent peeling. Other physical agents, such as x-ray, heat, chemicals, and trauma, also produce inflammation of the skin followed by exfoliation. In bacterial infection, of which scarlet fever is a fine example, scaling may be an end result of erythema. Whether infection is causative in this child, I don't know, but I think not.

Another cause of exfoliative dermatitis is

Table I. Relation of body weight to plasma protein therapy

Day	*Weight*	*Albumin*	*Hemoglobin*	*Treatment*
2	6 pounds, 8 ounces	2.9	6.7	
3				Blood (60 c.c.)
4	6 pounds, 8 ounces		15.2	Albumin (5 Gm.)
5	6 pounds, 13 ounces			
6				Albumin (5 Gm.)
7	7 pounds, 2 ounces		11.8	
8	6 pounds, 12 ounces	3.2		Albumin (5 Gm.)
9	6 pounds, 4 ounces			Albumin (5 Gm.)

allergy. Of the various kinds of hypersensitivity which give rise to inflammation and scaling, drug sensitivity is most common.

The fourth group of conditions associated with scaling comprises benign dermatoses, such as psoriasis, atopic eczema, seborrheic dermatitis, which, if sufficiently provoked, may give rise to an exfoliative process. At one point, I thought that this patient might have had very early psoriasis. There seems to be no strong reason to consider the harlequin fetus of congenital syphilis in this case.

Finally, we come to the group of exfoliative dermatitides of unknown cause in the neonatal period, and I suppose we will have to put this case into that group. In infancy, of course, we think of Ritter's disease and Leiner's disease. To many of us it still remains unclear as to just what these represent. Ritter's disease[1] is called dermatitis exfoliativa neonatorum. It begins rather abruptly at about 2 weeks of age and in addition to the redness and scaling there is a tendency to blister, which this child did not have. Nikolsky's sign is present (this means that the epidermis may be stripped off very easily just by laying your hand on it and moving it sideways). This was not present in this patient. Diarrhea and other systemic signs may occur very quickly in these patients, and death results, seemingly, from bacterial infection. Some patients apparently can be saved by antibiotic therapy. Original accounts of this condition indicated that it may occur in epidemic form. Leiner's disease[2] is very similar. It develops later, perhaps at 4 weeks of age. The alternate descriptive term is "erythroderma desquamativa." The lesions are present on the buttocks, without blistering, and tend to spread to the seborrheic areas. One gets the impression that this may be related to seborrheic dermatitis. These patients may have gastrointestinal symptoms, but they do not die as often as children with Ritter's disease. So, where do we stand? While Ritter's and Leiner's diseases may be of bacterial origin or variants of seborrheic dermatitis, their essence is not understood. We must get fresh information from long-range clinical studies, application of modern biochemistry, and from the newer approaches of basic science.

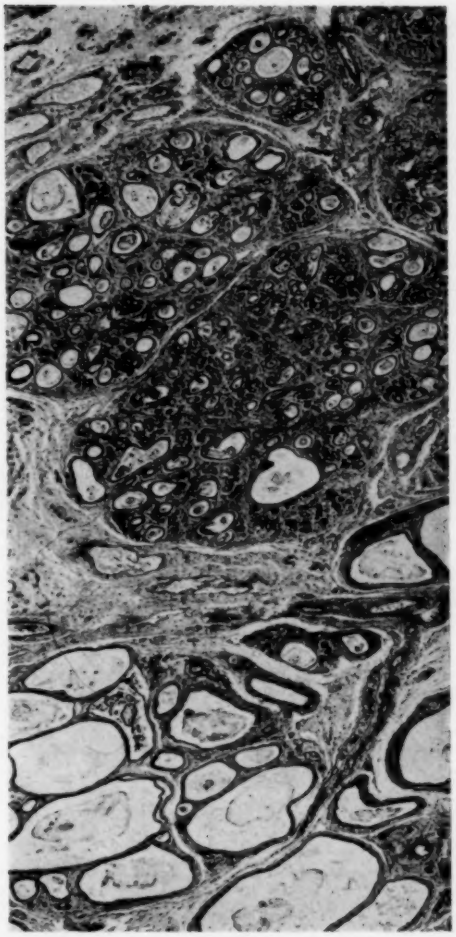

Fig. 4. Severe cystic changes involve pancreas. (Hematoxylin and eosin. Original magnification ×120.)

This patient may have had an unusual nutritional disorder. It is much more difficult, in clinical practice, to document specific or bizarre nutritional imbalance than it is in laboratory animals, in which deficiencies of unsaturated fatty acids, vitamins, or amino acids may be associated with erythema and scaling. Dr. Janeway, who had had opportunities to see deficiency states throughout the world, might like to comment.

DR. CHARLES A. JANEWAY. There is super-

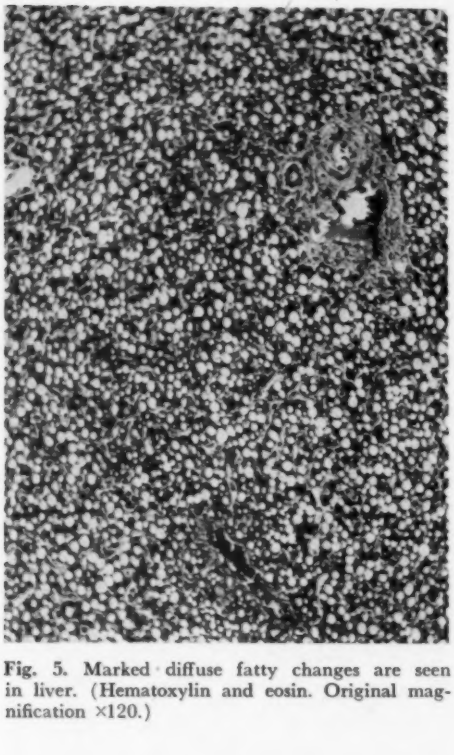

Fig. 5. Marked diffuse fatty changes are seen in liver. (Hematoxylin and eosin. Original magnification ×120.)

ficial clinical resemblance between this infant and those babies who have primary nutritional deficiency while nursing when the mothers produce a good volume of milk which contains deficient or poor quality protein or in which the caloric concentration is inadequate. It seems that under these circumstances the babies will continue to grow but because of unbalanced nutrition as well as the various environmental and social factors which go along with these things, they develop infections and perhaps diarrhea, both as result and as contributing factors in the development of such syndromes.

DR. GRIESEMER. May we have the x-ray report from Dr. Harris?

DR. G. B. C. HARRIS. The first x-ray on this child shows some degree of hyperaeration in the lungs with a little irregularity, but nothing very specific (Fig. 2). A few days later a second film shows dramatic change, with complete atelectasis and probably some infiltration in the right middle lobe (Fig. 3). A catheter in the trachea suggests some difficulty at the time. On the next day, there is spread of this disease to the left upper lobe. There has been quite an increase in the degree of edema evident in the 2 films, as well. In the final film, there is little change although there may be slight cardiac enlargement. We would not be able to arrive at any specific diagnosis, although the appearance is something that can be seen in cystic fibrosis. Of course, it could also occur with aspiration or with other forms of nonspecific pneumonia.

DR. JANEWAY. Generally speaking, staphylococcal pneumonia shows more reaction in the pleura, often with pleural effusions, pneumothorax, bleb formation, or pneumatocele, which this patient did not have.

DR. GRIESEMER. Finally, I think we would like to hear Dr. Shwachman's comment

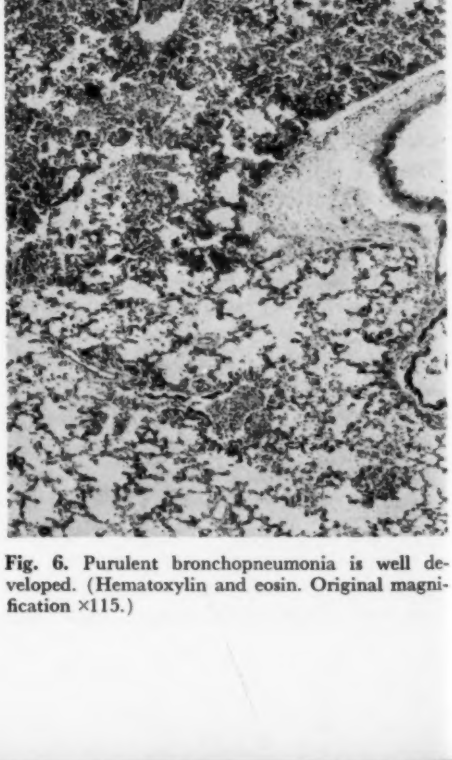

Fig. 6. Purulent bronchopneumonia is well developed. (Hematoxylin and eosin. Original magnification ×115.)

about the possibility of cystic fibrosis in this child.

DR. HARRY SHWACHMAN. I am afraid that I have no precise explanation. About 10 years ago we had our first experience with a baby with cystic fibrosis and severe edema who presented a clinical picture very similar to that of today's patient. The child recovered and is doing quite well. In the past 4 to 5 years we have seen a number of new symptoms and syndromes which, in the past, went unrecognized as early manifestations of cystic fibrosis. Unexplained hypoproteinemia occurring in newborn infants is one of these conditions. We have now seen 6 such patients and this child is the only one who has died. In the literature there are a number of reports of unexplained hypoproteinemia in the neonatal period. Dr. B. Vahlquist,[3] who reported such a case, was one of the first to recognize a possibility of pancreatic insufficiency, a condition later proved to exist on the basis of cystic fibrosis.

Many combinations of symptoms may occur. In a number of patients anemia is severe and in other cases Dr. Shahidi and I have demonstrated deficiency of ceruloplasmin, as well as other plasma proteins. Hypoproteinemic edema occurs and is the kind of edema that can be altered by changing position. We believe that the low serum protein is related to a lack of pancreatic enzymes. However, many patients have had inadequate protein by mouth, since they may present feeding problems or may have had a slight rash which suggests a milk allergy. The pediatrician, therefore, changes to other food sources, and not all sources seem to supply equally good protein requirements.

In the baby under discussion today, there was extensive exfoliative dermatitis, and I wonder if vitamin deficiencies played a role. In kwashiorkor, I think the skin lesions occur much later. One may question the adequacy of the mother's prenatal diet in this particular instance, since we know the family quite well and are aware of the fact that many pregnant women have an unbalanced or inadequate diet.

Insofar as the establishment of the diagnosis of cystic fibrosis is concerned, I think the clinical data and course of this baby are sufficient to establish a diagnosis. Our attempts to make the diagnosis on laboratory grounds were not entirely successful because this baby did not sweat very much. The iontophoresis test gave us one half of the 40 mg. of sweat needed for accurate analysis. I think we are successful in obtaining sufficient material for analysis about 80 per cent of the times we try it,

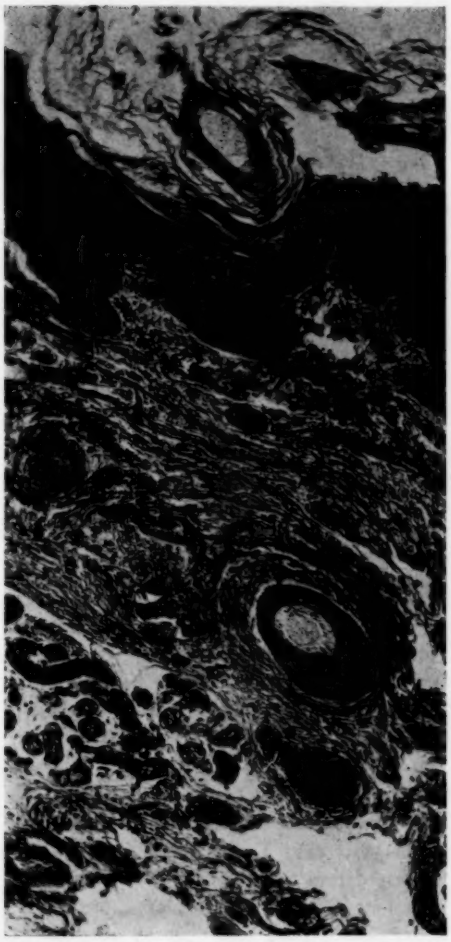

Fig. 7. Hyperkeratosis, acanthosis, and poorly developed sebaceous glands involve skin of scalp. (Hematoxylin and eosin. Original magnification ×130.)

and Dr. Elian has been successful even in 1-day-old babies. The values of sweat electrolytes in babies 1 and 2 days of age are normally higher than they are at 5 or 6 days of age, but not in the range that would make one suspect cystic fibrosis.

A number of stools were examined for proteolytic activity, and 1 was positive; 3 were negative. I should disregard the one that was positive and pay more attention to the 3 that were negative. If this child had not been so ill, we would have tried duodenal intubation as well as a sweat test. The sweat test is of little value, in an absolute way, in babies under 5 to 6 weeks of age. Therefore, we are not absolutely sure, from laboratory evidence, that this child had cystic fibrosis. There is no doubt about the diagnosis from the point of view of clinical history, positive family history, and course of the disease.

One other point about the distribution of fractions of the serum proteins. There was a high gamma globulin, in spite of the very low total protein. This might mean 2 things —infection and, I assume, more severe liver disease than we ordinarily see in babies with cystic fibrosis who die at this age. I do not believe that the amount of protein given was enough to produce pulmonary edema.

DR. VAWTER. Dr. Shwachman, can one expect to find decreased proteolytic enzymes in the stool, in the presence of severe protein deficiency? Would one have to examine the patient after his protein had come back toward normal before this would be a good diagnostic test?[4]

DR. SHWACHMAN. I don't think we can answer this from our own experience, since protein deficiency is not a common condition in patients at The Children's Hospital. I think this can be answered in countries such as South Africa or India and some other areas where nutritional diseases with hypoproteinemia are quite common.

We do know that such tests, done in patients with kwashiorkor, may be normal, and that pancreatic enzymes may be decreased in the stool. Whether the latter is a result of single mass protein deficiency (which is perhaps most likely) or of more specific derangements, is not known, as far as I am aware. Finally, I should say that this is only the second time that I have seen such a severe skin lesion, although it is true that all of our patients with hypoproteinemia have had some skin involvement, but generally considerably milder.

DR. VAWTER. Now you all have a clear idea of the problems confronting the pathologist in such a case.

The pathologic lesions which were found consisted of typical, severe cystic fibrosis of the pancreas (Fig. 4) with marked fatty liver (Fig. 5), and pulmonary hyperventilation, and purulent bronchopneumonia. Recent focal myocardial degeneration might have been anoxic or possibly nutritional in origin. Splenic, thymic, and lymphoid depletion such as accompanies malnutrition, was not associated with failure of leukopoiesis in marrow, whereas erythropoiesis seemed quite deficient.

I think it wisest, in view of what has been said, to confine comments on the skin to a description of the findings, together with a few speculative comments. Whereas there was great progress toward healing, a sort of geographic or "crazy pavement" scaling process persisted to some extent, especially on trunk and scalp, but there were no vesicles or oozing surfaces. Cheilosis was present. The tongue protruded, probably as a result of persisting edema, which was quite prominent.

Microscopically, in contrast to the atrophy and thinning of epidermis seen in total starvation, there was a thickening of epidermis (or acanthosis) with much scale (or hyperkeratosis) suggesting that of the 2 mechanisms for desquamation, mentioned by Dr. Griesemer, overproduction of biologic material was present here. Despite this and the normal appearance of the sweat glands, there was generally considerable atrophy of sebaceous glands. This latter lesion occurs in the late stages of a variety of laboratory nutritional dermatoses, riboflavin deficiency being one of the most prominent. Although there were a few dying polymorphonuclear

leukocytes in superficial keratin crusts overlying the epidermis, there was no cellular infiltration in epidermis or dermis where the principal sign of reactivity is in a mild dilation of blood vessels, together with edema, so that evidence for continuing superificial infection is lacking at this time.

In summary, we have an infant with *cystic fibrosis, severe hypoproteinemia, anemia* and *edema,* and *exfoliative dermatosis.* I had wondered, when originally studying this problem, what the importance of the long-standing desquamating process might be in the development of the deficiency syndrome. It now seems clear that multiple factors were probably in operation, but I would like to point out that keratin contains large quantities of 4 indispensable amino acids[5]: arginine, lysine, histidine, and cystine, deficiencies of which produce specific effects in the laboratory, some of which might have been pertinent here. In this regard, lysine might be of the most interest since it has a function in the conversion of acetate to glutamate. It is a heme precursor and a precursor of arginine. It acts as a lipotropic factor in the presence of low-protein diet, and it has something to do with coloration and development of hair.

REFERENCES

1. Lee, H. F., Wilson, R. B., Brown, C. E., and Reed, T. P.: Impetigo and Acute Infectious Exfoliative Dermatitis of the Newborn Infant (Ritter's Disease), J. Pediat. **41:** 159, 1952.
2. Leiner, C.: Erythroderma Desquamativa (Universal Dermatitis of Children at the Breast), Brit. J. Child. Dis. **5:** 244, 1908.
3. Bille, B. S. V., and Valquist, B.: Idiopathic Hypoproteinaemia Versus Hypoproteinaemia Due to Pancreatic Disfunction, Acta paediat. **44:** 435, 1955.
4. Veghelyi, P. V.: Fifth International Congress of Pediatrics, Acta paediat. **36:** 128, 143, 1948.
5. Greenberg, David M., editor: Amino Acids and Proteins, Springfield, Ill., 1951, Charles C Thomas, publisher, p. 260.

COMMENTS ON CURRENT LITERATURE

Heterozygous carriers in Wilson's disease

THREE basic features comprise the clinical syndrome known as Wilson's disease or hepatolenticular degeneration: extrapyramidal neurological symptoms, cirrhosis of the liver, and a Kayser-Fleischer ring in the cornea. The corneal defect, a brownish green ring at the periphery in the region of Descemet's membrane is seen in about 80 per cent of the patients. In one reported instance the patient sought medical advice because of difficulty in dark adaptation and night blindness, and in this case the corneal ring was the only significant physical finding.[1] Neither liver nor central nervous system damage was observed. Essentially the condition is characterized by an inherent inability to synthesize the copper-binding protein, ceruloplasmin, with resultant accumulation of copper in the body, especially in the liver and in the brain. Deficiency in an enzyme-apoenzyme system is presupposed at the present time. Urinary excretion of copper is increased, and there is a marked increase in the copper content of many organs and tissues.

Ceruloplasmin is described as an alpha globulin which has oxidase activity in which the blue color is due to a copper content of 8 atoms per molecule. Normally this protein fraction makes up about 0.5 per cent of the total serum proteins, being of the order of 30 mg. of ceruloplasmin per 100 ml. of serum. In Wilson's disease there may be a marked reduction in the concentration of this copper-binding serum protein. As much as 60 to 80 per cent of the serum copper is not bound to ceruloplasmin, as compared to the situation in normal persons in whom only about 10 per cent of the copper is not protein bound. While the precise mechanism is not completely understood as yet, it is thought that the increased concentration of loosely bound, nonceruloplasmin copper in the serum permits the accumulation of copper in the tissues and results in the increased excretion of copper in the urine. Recent studies indicate that there is in addition an excessive absorption of copper from the gut. In time the copper content increases in the cirrhotic liver and in the degenerating lenticular nucleus of the brain. Characteristically the degenerative process involves the basal ganglia.

In most instances the disease becomes manifest clinically in the latter part of childhood, marked by a rigidity of the skeletal musculature and by involuntary movements. There may be severe emotional instability and convulsive laughter without apparent provocation. The facial appearance is usually one of constant smiling; the speech becomes jerky and irregular. While the involuntary movements may be of any type, tremors involving the upper extremities to a greater degree than the lower ones are characteristic. Usually the tremor is present at rest, as in paralysis agitans, but it is most marked in association with voluntary movements. In older children, as a rule, the he-

patic symptoms are not obvious; liver function tests are not of particular diagnostic value; however, the cephalin flocculation test is often positive. In infants and young children, on the other hand, the presenting symptoms may be those of hepatic disease, and in this age group the diagnosis of hepatolenticular degeneration is a possibility in unexplained cirrhosis. In the adolescent patient, tremor is commonly the initial sign and is often diagnostic in type, particularly if at rest it resembles the Parkinsonian tremor, and it becomes on purposive movement a marked ataxia with choreiform components. This characteristic gross tremor involving the upper extremities has been described as suggestive of "flapping" (Fluegelschlagen). While the duration of the disease varies widely, the course in the absence of treatment is invariably progressive with associated mental changes.[2]

In recent years physiologic studies and investigations employing radioactive copper (Cu^{64}) have led to a better understanding of Wilson's disease. On the premise that the symptom complex in this condition stems from copper accumulation in the body, therapy has been directed toward removal of the excess copper in order to arrest the progress of the disease before irreversible damage occurs in the cerebral cortex and the basal ganglia. Several chelating agents have been used with considerable success; notably British Anti-Lewisite (BAL) and Versene, combined in some instances with high protein intake and potassium sulfide, or a form of carboresin after meals in an attempt to reduce the absorption of copper from the gut. However, the usefulness of BAL is limited in many patients by severe toxic manifestations and growing resistance.[2]

More recently the sulfhydryl amino acid, penicillamine (dimethyl cysteine), has been shown to be of value in the removal of copper from patients with Wilson's disease.[3-5] Walshe[3, 4] has described its use, its rationale, and compared its effectiveness with that of BAL. Penicillamine hydrochloride can be synthesized from penicillin, is stable in the reduced form, can be administered orally or parenterally, is extremely soluble, and is excreted rapidly by the kidney. In some cases remarkable improvement has followed its use.

That the disease has a high familial incidence was noted by Wilson,[6] himself, in his classic description in 1912. Frequently more than one sibling in a family is affected. In about one third of the cases investigated by Bearn[2, 7] the parents were known to be cousins. The high incidence of the condition among siblings, both boys and girls, children of parents free of the disease, together with known consanguinity in some affected families has made an autosomal recessive genetic pattern seem likely. This implies perpetuation of the recessive trait not only by those affected but by parents who are heterozygous carriers, and who do not show clinical manifestations of the disease. According to classic Mendelian concepts such heterozygous individuals would be indistinguishable from persons homozygous for the normal gene. In recent years, however, many reports indicate that heterozygous carriers of recessive genes can be identified by appropriate biochemical or hematologic studies, as in sickle cell anemia, where the sickle cell trait is identifiable; as in a number of inborn errors of metabolism: phenylketonuria, galactosemia, and acatalasemia, in all of which carriers of the recessive gene can be distinguished by appropriate tests.[8]

For a number of years Scheinberg and Sternlieb and their associates[9] have been interested in various aspects of Wilson's disease. A recent publication by this group[10] concerns the detection of the heterozygous individual and the carrier state in this condition. The fact that almost invariably the parents of children with Wilson's syndrome are themselves free of the disease led these authors to study several available families with a view to carrier identification. In some of these families more than one child was affected. The authors' reasoning was based on reports which indicate that heterozygous carriers can be identified biochemically in a number of hereditary conditions which follow the recessive genetic pattern, particu-

larly the inborn errors of metabolism.[8] "Quantitive or qualitative changes, or both, in the protein whose synthesis the abnormal gene is assumed to govern may be demonstrable . . . or there may be abnormal metabolism of elements or compounds, the usual metabolism of which is dependent on adequate concentrations of the normal protein."[10] Previous studies with Cu^{64} had shown that a much lower than normal incorporation of Cu^{64} into ceruloplasmin can be demonstrated in patients with Wilson's disease. Following the ingestion of cupric^{64} acetate or sulfate, there is within 1 to 2 hours a sharp rise and fall in the serum concentration of Cu^{64} in all individuals. Normal persons show a slow secondary rise in serum Cu^{64} concentration, due to Cu^{64} which has been incorporated into ceruloplasmin. Patients with Wilson's disease do not show this secondary rise.

In one of the initial investigations the authors encountered a normal parent of one of the patients with Wilson's disease who showed a very low level of Cu^{64} incorporation into ceruloplasmin. This observation spurred the testing of other parents and of a comparable control group. Nineteen individuals with no family history of Wilson's disease comprised this control group. Seven patients with Wilson's disease were studied at the same time; these by definition possess 2 recessive hereditary factors for the disease. Nineteen parents of patients with Wilson's disease were test subjects as possible heterozygous carriers of the recessive trait. Forty-five relatives of patients also served as test subjects; this group included unaffected siblings, half-siblings, aunts, uncles, grandparents, cousins, and half-nieces. The test procedure was arbitrarily standardized with respect to the copper load. A dose of 2.0 mg. of copper as cupric^{64} acetate or sulfate, with an activity of from 0.5 to 2.0 mc., was administered in a cup of milk to each individual following an 8-hour fast. The amount of radioactive copper was determined in a well-type scintillation counter at 1, 2, 4, 24, and 48 hours after ingestion. Radioactivity measurements obtained were corrected for physical decay with the use of a half-life of 12.8 hours for Cu^{64} as standard expected loss of radioactivity. The levels observed at 48 hours following ingestion are key figures since they represent essentially only that Cu^{64} which has been incorporated into ceruloplasmin, and indicate, therefore, the Cu^{64}-binding capacity of the ceruloplasmin. The initial peak of serum Cu^{64} concentration 1 to 2 hours following ingestion is considered a rough index of the amount of Cu^{64} absorbed from the gastrointestinal tract. The authors, therefore, employed as a tool the ratio of the serum Cu^{64} concentration at 48 hours to the serum Cu^{64} concentration at 1 to 2 hours. Patients with Wilson's disease show the expected initial sharp rise in Cu^{64} serum concentration within the first 2 hours after ingestion, followed by the expected fall, but do not show the secondary rise representing the ceruloplasmin-incorporated copper. Control individuals with normal ceruloplasmin show the secondary rise with the expected peak at about 48 hours. Parents of children with Wilson's disease and heterozygous collateral relatives show the initial rise and fall, with the secondary rise reaching a peak level at 48 hours which is intermediate between the 2 other groups. These results were consistently reproducible, and the authors consider the geometric means calculated from these figures statistically significant. They conclude that these "measurements of the incorporation of a load of copper^{64} into ceruloplasmin can provide a fairly reliable means of distinguishing heterozygotes for the Wilson's gene from individuals who are homozygous for its normal allele."[10]

Quantitative studies of the ceruloplasmin in heterozygous individuals were of particular interest in that the quantity of this serum protein appears to be adequate in heterozygotes but has lower copper-combining power, suggesting an abnormal form of ceruloplasmin. Several varieties of human ceruloplasmin have been described within the past year.[11] In summary the authors emphasize that the exact mechanism for the lowered copper-combining power of the serum in heterozygous individuals cannot be eluci-

dated on the basis of these preliminary studies. However, the observation concerning the heterozygous carrier state is in itself of interest since it "seems to provide another instance" in human heredity "wherein the presence of a single gene may be manifest biochemically."[10]

RUSSELL J. BLATTNER, M.D.

REFERENCES

1. Segal, P., Ruskowski, M., Berger, S., and Masaik, M.: Abortive Form of Wilson's Disease, Am. J. Ophth. **44:** 623, 1957.
2. Blattner, R. J.: Chelating Agents in the Treatment of Hepatolenticular Degeneration (Wilson's Disease), J. PEDIAT. **52:** 758, 1958.
3. Walshe, J. M.: Wilson's Disease: New Oral Therapy, Lancet **1:** 25, 1956; Penicillamine, A New Oral Therapy for Wilson's Disease, Am. J. Med. **21:** 487, 1956; Hepatolenticular Degeneration (Wilson's Disease), Brit. M. Bull. **13:** 1322, 1957; Current Views on the Pathogenesis and Treatment of Wilson's Disease, A. M. A. Arch. Int. Med. **103:** 155, 1959; Treatment of Wilson's Disease With Penicillamine, Lancet **1:** 188, 1960.
4. Osborn, S. B., and Walshe, J. M.: Effects of Penicillamine and Dimercaprol on Turnover of Copper in Patients With Wilson's Disease, Lancet **1:** 70, 1958.
5. Fister, W. P., Boulding, J. E., and Baker, R. E.: The Treatment of Hepatolenticular Degeneration With Penicillamine, With Report of Two Cases, Canad. M. A. J. **78:** 99, 1958.
6. Wilson, S. A. K.: Progressive Lenticular Degeneration: A Familial Nervous Disease Associated With Cirrhosis of the Liver, Brain **34:** 295, 1912.
7. Bearn, A. G.: Wilson's Disease: An Inborn Error of Metabolism With Multiple Manifestations, Am. J. Med. **22:** 747, 1957; A Genetical Analysis of Thirty Families With Wilson's Disease (Hepatolenticular Degeneration), Ann. Human Genet. **24:** 33, 1960.
8. Hsia, D. Y.-Y.: Medical Genetics, New England J. Med. **262:** 1172, 1222, 1273, 1318, 1960.
   Hsia, D. Y.-Y., Tannenbaum, M., Schneider, J. A., Huang, I., and Simpson, K.: Further Studies on the Heterozygous Carrier in Galactosemia, J. Lab. & Clin. Med. **56:** 368, 1960.
   Takahara, S., Hamilton, H. B., Neel, J. V., Kobara, Thos. Y., Ogura, Y., and Nishimura, E. T.: Hypocatalassemia: A New Genetic Carrier State, J. Clin. Invest. **39:** 610, 1960.
9. Scheinberg, I. H., and Sternlieb, I.: The Pathogenesis and Clinical Significance of the Liver Disease in Hepatolenticular Degeneration (Wilson's Disease), M. Clin. North America **44:** 665, 1960.
10. Sternlieb, I., Morell, A. G., Bauer, C. D., Combes, B., de Bobes-Sternberg, S., and Scheinberg, I. H.: Detection of the Heterozygous Carrier of the Wilson's Disease Gene, J. Clin. Invest. **40:** 707, 1961.
11. Morell, A. G., and Scheinberg, I. H.: Heterogeneity of Human Ceruloplasmin, Science **131:** 930, 1960.

Books

The Development of the Infant and Young Child (Normal and Abnormal).
R. S. Illingworth, M.D., Edinburgh and London, 1960, E. & S. Livingstone, Ltd. (in U. S., Williams & Wilkins Company, Baltimore), 313 pages. Price $6.50.

This small volume contains lucid, concise outlines and discussions of early development. It says neither too much nor too little for the author's purpose, which is to tell the doctor in simple language what can be learned with a minimum of equipment, space, and training. Variability, limitations, and pitfalls of developmental examinations are discussed in clear, uncluttered prose. The photographs and their captions are to the point. Physical development (weight, height, etc.) is omitted. This book is for those who have attempted to read other texts on pediatric neurology or on developmental diagnosis and found them hypnotic rather than informative.

HENRY W. BAIRD, III, M.D.

World Health Organization Monograph Series No. 40.
Child Guidance Centres, World Health Organizations, D. Buckle and S. Lebovivi, editors, Geneva, 1960, Palais des Nations, 127 pages.

This 127 page monograph is a summary of lectures given in the Seminar at Lausanne from Sept. 18 to 29, 1956, under the auspices of the Regional Office for Europe of the World Health Organization.

In general, it is a fairly complete assembly of the facts discussed on the operation of a child guidance center, mechanically and theoretically. The first 10 chapters deal primarily with administrative matters, case-history taking, training of professional workers, etc., diagnosis, and classification of disorders. A very useful appendix, pages 107 to 126, discusses psychoanalytical concepts in child development. Section on training of professional workers, psychologists, is quite complete and reflects present-day philosophy except for possibly a lack of emphasis on supervised experience. I feel that the treatment section is too heavily psychoanalytic but the functions of the psychologists are well spelled out. Classification of behavior disorders suffers from the usual difficulties, overlapping categories, etc., but it probably is as satisfactory as any present-day classification.

In general, this is a worthwhile elementary type book for someone interested in establishing a child guidance center.

HOMER F. WEIR, M.D.

Biology of Pyelonephritis.
Edward L. Quinn, M.D., and Edward H. Kass, M.D., Ph.D., editors, Boston, 1960, Little, Brown and Co., 708 pages. Price $18.00.

This book derives from the Henry Ford Hospital symposium of the same name, held in Detroit, Oct. 8 to 10, 1959, at which the work of 80 investigators was presented.

The title indicates the contents of this book rather inadequately, since many aspects of renal disease and function are thoroughly covered, including water and electrolyte physiology, blood pressure regulation, immunologic mechanisms, etc. It is well written in a clear, informal style, and carefully edited; the illustrations are well done also.

The only significant criticism relates to the failure to include data specifically related to pyelonephritis in infants and children. Despite this omission, the book should be of interest to all pediatricians.

DANIEL S. FLEISHER, M.D.

Correspondence

To the Editor:

I would like to call attention to the problem of digitalization in the newborn infant.

There is increasing evidence that premature infants and full-term neonates are more sensitive to digitalis than older infants. One might indeed theoretically expect such an increased sensitivity to digitalis in these infants because of hepatic and renal immaturity.

I have seen and also been apprised by colleagues of other instances of digitalis toxicity in both premature infants and term neonates; these babies were digitalized with recommended doses of digoxin (.075 to .08 mg. per kilogram per 24 hours).[1-3]

One does find an occasional reference[4, 5] suggesting that there is an increased sensitivity to digitalis in the newborn infant but without documentation of the evidence for this. Recently, however, and as far as I am aware, for the first time a study has been recorded on this subject. Levine and Blumenthal[6] report that premature infants showed considerable toxicity to a digitalizing dose of .075 mg. of digoxin per kilogram per 24 hours and much less difficulty from two thirds this dose.

It is almost unnecessary to repeat the fact that there is considerable variation in sensitivity to digitalis at *all* ages. The standard recommended total digitalizing dose, as has been stressed, is only an "average digitalizing dose."[1, 2] Each patient should be given the calculated "average digitalizing dose" for his age in divided amounts and followed closely. This estimated total dose should always be readjusted if either toxicity or the desired therapeutic effect appears before the full dose is given. On the other hand, more than this "average" total dose may at times be required.

This principle of digitalization obviously should also be followed in the neonatal period. However, in view of the demonstration of increased sensitivity to digitalis in the premature infant and probably also in term neonates, it would seem advisable at this age to start with approximately two thirds of the "average digitalizing dose" recommended for older infants.

One could, therefore, estimate for digoxin .05 mg. per kilogram per 24 hours, intramuscularly, as an "average digitalizing dose" in the premature or term newborn infant; one half of the total is given first, then one fourth of the total in 6 to 8 hours, and one fourth again in another 6 to 8 hours. If, at this point, the desired effect is still lacking and there is no evidence of toxicity, the last dose can be repeated at 6 to 8 hour intervals before a maintenance dose is established.

It should be stressed that the above dosage figures apply only to digoxin, which, I believe, is the drug of choice for digitalization in infants and children.[3]

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REFERENCES

1. Sapin, S. O., Donoso, E., and Blumenthal, S.: Digoxin Dosage in Infants, Pediatrics **18**: 730, 1956.
2. Hauck, A. J., Ongley, P. A., and Nadas, A. S.: The Use of Digoxin in Infants and Children, Am. Heart J. **56**: 443, 1958.
3. Sapin, S. O.: Digitalis Therapy in Pediatrics, Quart. Rev. Pediat. **15**: 41, 1960.
4. Schaffer, A. J.: Diseases of the Newborn, Philadelphia, 1960, W. B. Saunders Company, p. 293.
5. Neill, C. A.: Recognition and Treatment of Congestive Heart Failure in Infancy, Mod. Concepts Cardiovas. Dis. **27**: 507, 1959.
6. Levine, O. Robert, and Blumenthal, Sidney: Digoxin Dosage in Premature Infants, presented at the Soc. for Pediat. Res. Meet. Atlantic City, New Jersey, 1961, May 4 to 5.

*News and notes**

The American Board of Pediatrics, Inc., announces that the following diplomates were certified at Atlantic City, New Jersey on April 28 to 30, and May 1 to 2, 1961:

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The Pediatric Section of Kings County Medical Society, Brooklyn, New York, announces the election of its officers for the year 1961-1962 as follows: President, Ben Zweifler, M.D.; Vice President, Ernest T. Heffer, M.D.; Secretary, Hyman Leiberman, M.D.; and Treasurer, Richard D. Turin, M.D.

The American Friends of Vietnam (Room 204, 4 W. 40th Street, New York 18) are soliciting assistance for the University of Hue, in the free Republic of Vietnam. This University, whose enrollment now numbers 1,192 students, has this past fall opened its new School of Medicine, which offers a 7-year program in all fields of medical education. Pharmaceutics, reference books, and professional journals (including back numbers of this JOURNAL) are sought; such gifts are tax deductible and should be sent to the above address, from which they will be shipped to the University. Donors will receive formal receipts acknowledging the value of the contribution. (The C. V. Mosby Company has kindly offered to send copies in stock of its journals for 1960 and 1961.)

The Chicago Pediatric Society announces the election of the following officers for the year 1961-1962: President, Dr. James A. Conner, of Hinsdale; Vice-President, Dr. I. Pat Bronstein; Secretary, Dr. Joseph R. Christian; Treasurer, Dr. Harry L. Faulkner; Editor, Dr. Howard S. Traisman; and Executive Committee, Dr. Noel G. Shaw, Dr. Raymond F. Grissom, and Dr. Matthew M. Steiner.

On Sept. 18 to 21, 1961, the **Minnesota Post-Graduate program** will be presented in Minneapolis. The annual McQuarrie Lecture will be delivered by Dr. Lawson Wilkins on Sept. 20, 1961; other speakers include Drs. Ralph Platou, Edward Press, Robert Blizzard, Alvin B. Hayles, T. C. Evans, Harry Ginsburg, Robert Good, and Lewis Wannamaker. For reservations and more detailed information, write the Center for Postgraduate Education, University of Minnesota, Minneapolis, Minn.

A training program designed to lead to an academic career in pediatric endocrinology and metabolism is offered by the division of Endocrinology and Metabolism, Department of Pediatrics, University of California at Los Angeles. Summer traineeships are available to medical students who have completed 2 years of medical school. For postdoctoral candidates who have completed Pediatric Board residency requirements, a 2-year training period is offered. Stipends depend on need and prevailing salary scales. Address Dr. H. D. Mosier, Program Director, Department of Pediatrics, UCLA Medical Center, Los Angeles 24, Calif.

The **Queens Pediatric Society of Long Island** announces the election of the following officers for 1961-1962: President, Elmer E. Amerman, M.D., of Jackson Heights; President-Elect, Joseph Stein, M.D., of Jamaica; Treasurer, John DeHoff, M.D., of Flushing; and Secretary, Alfred L. Abrams, M.D., of Lake Success. Meetings are held at The Seminole Club in Forest Hills on the second Tuesday of October, December, February, and April.

The Society for Pediatric Research announces the election of the following officers and new members at its meeting in Atlantic City on May 4, 1961:

Officers elected for 1962:

President, Frederick C. Robbins, Cleveland, Ohio
Vice-president, Julius B. Richmond, Syracuse, New York
President-elect, Lytt I. Gardner, Syracuse, New York
Secretary-Treasurer, Clark D. West, Cincinnati, Ohio

Members of Council:

Floyd W. Denny, Jr., 1962 to 1964, Chapel Hill, North Carolina
Nathan J. Smith, 1962 to 1964, Madison, Wisconsin
David Gitlin, 1962 to 1963, Boston, Massachusetts
Sydney Segal, 1962 to 1963, Vancouver, Canada
Edna Sobel, 1962, New York, New York
Forrest Adams, 1962, Los Angeles, California

Newly elected members:

Peter A. M. Auld, Harvard University
Jay Bernstein, Wayne University
Ira K. Brandt, Yale University
Flossie Cohen, Wayne University
Robert L. Cranny, Albany University
Robert Fowler, Jr., Melbourne University
Hossein Ghadimi, Toronto University
Richard B. Goldbloom, McGill University
Robert J. Haggerty, Harvard University
Klaus Hummeler, University of Pennsylvania
George S. Husson, Syracuse University
John A. Kirkpatrick, Jr., Temple University
Martha L. Lepow, Western Reserve University
Charles C. Lobeck, Wisconsin University
Ernest E. McCoy, Vanderbilt University
Donald N. Medearis, Johns Hopkins Hospital
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Catherine A. Neill, Johns Hopkins Hospital
Milton H. Paul, Northwestern University
William J. Rashkind, University of Pennsylvania
William K. Schubert, Cincinnati University
Herbert C. Schwartz, Stanford University
David W. Smith, Wisconsin University
Gunnar B. Stickler, Mayo Clinic
Paul M. Taylor, Pittsburgh University
William G. Thurman, Tulane University
Peter Vlad, Buffalo University
Harold G. Worthen, Minnesota University
William C. Yakovac, University of Pennsylvania

The American Academy of Pediatrics has announced the winners of two awards: the Borden Award has been presented to Dr. Clement A. Smith, of Boston, Mass.; and the E. Mead Johnson Award has been won jointly by Dr. Lytt I. Gardner of Syracuse, N. Y., and Dr. Donald E. Pickering, of Portland, Ore. Papers by the 3 winners will be presented at the Academy's October meeting.

A continuation course entitled **Pediatrics for Specialists** will be presented at the Center for Continuation Study, University of Minnesota, in Minneapolis, Sept. 18 to 21, 1961. Address inquiries to Dr. W. Albert Sullivan, Jr., M.D., Director, Department of Continuation Medical Education, 1342 Mayo Memorial, University of Minnesota Medical School, Minneapolis 14, Minn.

On Oct. 26 to 28, 1961, the Department of Pediatrics of Baylor University and the Texas State Department of Health offer a **Pediatric Postgraduate Seminar** at **Texas Children's Hospital, Houston.** Speakers will include: Dr. Victor Najaar, Dr. Sam Bessman, Dr. Irving Schulman, Dr. Melvin Grumbach, and Dr. Edward Hon. For information address Dr. Russell J. Blattner, Chairman, Department of Pediatrics, Baylor University College of Medicine, Houston, Texas.

The **American College of Surgeons** announces a postgraduate course on **Recent Advances in Pediatric Surgery,** to be given at the Clinical Congress, Oct. 2 to 6, 1961, in Chicago. Speakers for the session on "The Infant and Child As a Surgical Patient" are H. William Clatworthy, Jr., Robert M. Smith, William R. Richardson, Harry C. Bishop, and Willis J. Potts. On the topic "Surgery of the Inguinal Area," the speakers will include William H. Snyder, Jr., Peter K. Kottmeier, Michel Gilbert, William L. Riker, John P. Smith, and Lorye E. Hackworth. The session on "Vascular Lesions" will be addressed by Richard L. Varco, Eugene F. Poutasse, E. Thomas Boles, Jr., William T. Mustard, Lester W. Martin, and George W. Holcomb, Jr. "Genitourinary Problems" will be discussed by John K. Lattimer, Irving A. Meeker, Jr., Alexander J. Michie, Albert J. Paquin, Jr., Eugene M. Bricker, and Mark M. Ravitch. Address inquiries to the American College of Surgeons, 40 East Erie Street, Chicago 11, Ill.; there is a charge of $10.00 for the course.

New officers of the **Los Angeles Pediatric Society** have been announced: President; Neil N. Litman, M.D.; Vice-President, Joan Hodgman, M.D.; and Secretary-Treasurer, Leslie M. Holve, M.D.

The Roger Williams General Hospital in Providence, Rhode Island, announces a **Symposium on Preventive Pediatrics** to be held on Oct. 11, 1961. The moderator will be Sydney S. Gellis, M.D.; participants include Drs. William Silverman, Kurt Isselbacker, W. Hardy Hendron, R. C. Eley, Trygve Gunderson, Adam J. Sortini, Richard Galdston, and Roswell Gallagher. Address inquiries to Harry E. Darrah, M.D., Director of Medical Education, Roger Williams General Hospital, 825 Chalkstone Avenue, Providence 8, R. I.

The Southern Society for Pediatric Research announces its second annual meeting to be held in Atlanta, Georgia, Nov. 17 to 18, 1961. Abstracts (300 words) must be sent before Sept. 10, 1961, to Dr. W. G. Thurman, Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia. Inquiries regarding registration should also be sent to him.

EDITOR'S COLUMN

Tuberculosis and tuberculosis-like diseases

THE observation reported in this issue by Boyd and Craig, which may eliminate cat-scratch fever as a clinical entity, adds another chapter to the long and varied history of tuberculosis and of the diseases which simulate it.

If one looks back merely to the first decade of the present century, human tuberculosis was then recognized as a relatively common, serious, acute, and chronic infection with a variety of clinical patterns and as a leading cause of death. With the adaptation of tuberculin as a skin testing medium by Escherich and in particular by Pirquet, it became evident that tuberculous infection was much more prevalent than had been recognized by clinical and even by pathologic studies. In some geographic areas, especially congested urban ones, the tuberculin test elicited positive dermal reactions in most all adults and in a significant percentage of children. It became apparent that the infection often occurred and healed with such subtlety that not even the acute phase was recognized clinically.

Further evidence of the very high incidence of tuberculous infection was subsequently provided when roentgen examinations of the chest became commonplace. Calcified pulmonary and mediastinal lesions were found to be quite common in adults and not uncommon in older children. Furthermore, rather extensive noncalcified lesions in the lungs and mediastinal lymph nodes in association with positive dermal tuberculin reactions were observed with sufficient frequency in children who were not clinically ill to indicate that the destructiveness of tuberculosis varied from person to person. Thus the concept, not wholly correct, of benignity of the first or primary tuberculous infection evolved. This concept was relatively more appropriate for children in the years from infancy to adolescence.

It became axiomatic: (1) that a positive tuberculin reaction was indicative of tuberculous infection, but not necessarily of the disease, (2) that calcification within the lungs or mediastinal lymph nodes should be considered as tuberculous in origin until proved otherwise, and (3) that the combination of a positive tuberculin reaction and roentgenographically demonstrable pulmonary and/or mediastinal calcifications was in essence a confirmation of the tuberculous origin of the calcified lesions.

None of these dictums can now be accepted unequivocally in the individual case.

Until quite recently, the tuberculin reaction was essentially unchallenged as an indicator of sensitization to the tubercle bacillus. Cross hypersensitivity to the different strains of *Mycobacterium tuberculosis*, especially to the human and bovine strains, has long been established. Within recent years, however, it has become evident that some of the other acid-fast bacteria are also antigenically related to the tubercle bacillus. How many of these there may be is not known, but several chromogenic and nonchromogenic types of mycobacteria have been identified as being antigenically related to *M. tuberculosis* and capable of producing disease in man.[1] These bacteria prior to

these observations had been considered saprophytes as far as man is concerned. Now it would seem that they may be responsible for rather widespread infection, especially in certain geographic areas. Further, these infections may mimic at least some of the tuberculous lesions, even though they appear to be less virulent. Palmer and colleagues[2] have demonstrated clearly the cross sensitivity between the antigens of the tubercle bacillus and of the so-called Battey bacterium, a nonphotochromogenic strain of mycobacteria. From their observations, they inferred that, when there were reactions to both skin testing materials, the larger reaction probably identified the infecting agent. Thus it may become necessary under given circumstances to employ several "tuberculin" skin tests. In addition to the tuberculin of *M. tuberculosis,* similar testing material from one or more of the mycobacteria currently inadequately classified as atypical or anonymous acid-fast bacilli may be essential to identify the infecting agent when the tuberculin test is positive, and some doubt exists whether the infection may be caused by one of several acid-fast bacilli.[3]

During the decade of the 1930's, it became increasingly evident that pulmonary and mediastinal calcifications occurred with some frequency in association with negative tuberculin reactions. These observations were made principally in the Ohio River basin.[4] In the considerations of whether these lesions represented healed tuberculous lesions and loss of allergy to tuberculin or whether they represented the healed lesions of some nontuberculous infection, C. E. Smith[5] suggested that histoplasmosis be considered as a possible cause. Subsequently, Christie and Peterson[6] and Palmer[7] separately demonstrated that such was the case. They further showed that histoplasmosis was a widespread infection in the eastern central states of the United States and that, like tuberculosis, the infection was most often not recognized clinically. In fact, in this area, histoplasmosis is probably a much more common cause of intrathoracic calcification than is tuberculosis.

Thus, when the dermal reactions to tuberculin and histoplasmin are both positive in an individual with pulmonary and/or mediastinal calcifications, there is no way to identify the origin of the calcified lesions.

It is apparent that the history of tuberculosis is not limited to infections by the tubercle bacillus. Coccidioidomycosis and histoplasmosis are the outstanding examples of diseases which have been separated from what might be termed the tuberculosis pool. But the demonstration in Korea that paragonimiasis[8] could simulate tuberculosis clinically is another of the significant contributions which has helped to clarify the tuberculosis picture. On the other hand, there continue to be such etiologically unidentified diseases as Hodgkin's and sarcoidosis in which many attempts to relate them to tuberculosis have failed.

Now interest is centered on the epidemiologic and clinical patterns of human infections by the mycobacteria which bear some antigenic relationship with the tubercle bacillus. One may anticipate that there will be new chapters and perhaps unexpected ones related to tuberculosis and tuberculosis-like diseases.

W. E. N.

REFERENCES

1. Davis, S. D., and Comstock, G. W.: Mycobacterial Cervical Adenitis in Children, J. PEDIAT. **58:** 771, 1961.
   Chapman, J. S., and Guy, L. Ruth: Scrofula Caused by Atypical Mycobacteria, Pediatrics **23:** 323, 1959.
2. Palmer, C. E., Edwards, Lydia B., Hopwood, Louise, and Edwards, Phyllis Q.: Experimental and Epidemiologic Basis for the Interpretation of Tuberculin Sensitivity, J. PEDIAT. **55:** 413, 1959.
3. Nelson, W. E.: The Role of Tuberculin in the Detection of Tuberculous Infection, J. PEDIAT. **55:** 541, 1959.
4. Nelson, W. E., Mitchell, A. G., and Brown, E. W.: Intracutaneous Tuberculin Reaction Associated With Calcified Intrathoracic Lesions, Am. Rev. Tuberc. **37:** 311, 1938.
5. Smith, C. E.: Personal communication.
6. Christie, A., and Peterson, J. C.: Pulmonary Calcification in Negative Reactors to Tuberculin, Am. J. Pub. Health **35:** 1131, 1945.
7. Palmer, C. E.: Nontuberculous Calcification and Sensitivity to Histoplasmin, Pub. Health Rep. **60:** 513, 1945.
8. Yun, D. J.: Paragonimiasis in Children in Korea, J. PEDIAT. **56:** 736, 1960.